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TITLE OF THE INVENTION (280 characters max)

PROTEIN C AND ENDOTHELIAL PROTEIN C RECEPTOR POLYMORPHISMS AS INDICATORS OF PATIENT OUTCOME

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PROTEIN C AND ENDOTHELIAL PROTEIN C RECEPTOR POLYMORPHISMS AS INDICATORS OF PATIENT OUTCOME

FIELD OF THE INVENTION

The field of the invention relates to the assessment and/or treatment of patients with an inflammatory condition.

BACKGROUND OF THE INVENTION

Genotype has been shown to play a role in the prediction of patient outcome in inflammatory and infectious diseases (MCGUIRE W. et al. *Nature* (1994) 371:508-10; NADEL S. et al. *Journal of Infectious Diseases* (1996) 174:878-80; MIRA JP. et al. *JAMA* (1999) 282:561-8; MAJETSCHAK M. et al. *Ann Surg* (1999) 230:207-14; STUBER F. et al. *Crit Care Med* (1996) 24:381-4; STUBER F. et al. *Journal of Inflammation* (1996) 46:42-50; and WEITKAMP JH. et al. *Infection* (2000) 28:92-6). Furthermore, septic and non-septic stimuli such as bacterial endotoxin and cardiopulmonary bypass (CPB), respectively, activate the coagulation system and trigger a systemic inflammatory response syndrome (SIRS). Protein C and endothelial cell protein C receptor (EPCR) both play a role in the inflammatory response.

Protein C, when activated to form activated protein C (APC), plays a major role in three biological processes or conditions: coagulation, fibrinolysis and inflammation. Acute inflammatory states decrease levels of the free form of protein S, which decreases APC function because free protein S is an important co-factor for APC. Sepsis, acute inflammation and cytokines decrease thrombomodulin expression on endothelial cells

resulting in decreased APC activity or levels. Septic shock also increases circulating levels of thrombomodulin, which is related to increased cleavage of endothelial cell thrombomodulin. Another mechanism for decreased APC function in sepsis is that endotoxin and cytokines, such as TNF-α, down-regulate endothelial cell protein C receptor (EPCR) expression, thereby decreasing activation of protein C to APC. Severe septic states such as meningococcemia, also result in protein C consumption. Depressed protein C levels correlate with purpura, digital infarction and death in meningococcemia.

Protein C is also altered in non-septic patients following cardiopulmonary bypass (CPB). Total protein C, APC and protein S decrease during CPB. Following aortic unclamping (reperfusion at the end of CPB) protein C is further activated so that the proportion of remaining non-activated protein C is greatly decreased. A decrease of protein C during and after CPB increases the risk of thrombosis, disseminated intravascular coagulation (DIC), organ ischemia and inflammation intra- and post-operatively. Patients who have less activated protein C generally have impaired recovery of cardiac function, consistent with the idea that lower levels of protein C increase the risk of microvascular thrombosis and myocardial ischemia. Aprotinin is a competitive inhibitor of APC, and is sometimes used in cardiac surgery and CPB. Aprotinin has been implicated as a cause of post-operative thrombotic complications after deep hypothermic circulatory arrest.

Septic and non-septic stimuli such as bacterial endotoxin and cardiopulmonary bypass (CPB), activate the coagulation system and trigger a systemic inflammatory response syndrome (SIRS). A decrease in protein C levels have been shown in patients with septic shock (GRIFFIN JH. *et al.* (1982) Blood 60:261-264; TAYLOR FB. *et al.* (1987) J. Clin. Invest. 79:918-925; HESSELVIK JF. *et al.* (1991) Thromb. Haemost. 65:126-129;

FIJNVANDRAAT K. *et al.* (1995) Thromb. Haemost. 73(1):15-20), with severe infection (HESSELVIK JF. *et al.* (1991) Thromb. Haemost. 65:126-129) and after major surgery (BLAMEY SL. *et al.* (1985) Thromb. Haemost. 54:622-625). It has been suggested that this decrease is caused by a decrease in protein C transcription (SPEK CA. *et al.* J. Biological Chemistry (1995) 270(41):24216-21 at 24221). It has also been demonstrated that endothelial pathways required for protein C activation are impaired in severe menigococcal sepsis (FAUST SN. *et al.* New Eng. J. Med. (2001) 345:408-416). Low protein C levels in sepsis patients are related to poor prognosis (YAN SB. and DHAINAUT J-F. Critical Care Medicine (2001) 29(7):S69-S74; FISHER CJ. and YAN SB. Critical Care Medicine (2000) 28(9 Suppl):S49-S56; VERVLOET MG. *et al.* Semin Thromb Hemost. (1998) 24(1):33-44; LORENTE JA. *et al.* Chest (1993) 103(5):1536-42). Recombinant human activated protein C reduces mortality in patients having severe sepsis or septic shock (BERNARD GR. *et al.* New Eng. J. Med. (2001) 344:699-709). Thus protein C appears to play an important beneficial role in the systemic inflammatory response syndrome.

The human protein C gene maps to chromosome 2q13-q14 and extends over 11kb. A representative *Homo sapiens* protein C gene sequence is listed in GenBank under accession number AF378903. Three single nucleotide polymorphisms (SNPs) have been identified in the 5′ untranslated promoter region of the protein C gene and are characterized as -1654 C/T, -1641 A/G and -1476 A/T (according to the numbering scheme of FOSTER DC. *et al.* Proc Natl Acad Sci U S A (1985) 82(14):4673-4677), or as -153C/T, -140A/G and +26A/T respectively by (MILLAR DS. *et al.* Hum. Genet. (2000) 106:646-653 at 651).

The genotype homozygous for -1654 C/ -1641 G/ -1476 T has been associated with reduced rates of transcription of the protein C gene as compared to the -1654 T/ -1641 A/ -1476 A homozygous genotype (SCOPES D. *et al.* Blood Coagul. Fibrinolysis (1995) 6(4):317-321). Patients homozygous for the -1654 C/ -1641 G/ -1476 T genotype show a decrease of 22% in plasma protein C levels and protein C activity levels as compared to patients homozygous for the -1654 T/ -1641 A/ -1476 A genotype (SPEK CA. *et al.* Arteriosclerosis, Thrombosis, and Vascular Biology (1995) 15:214-218). The -1654 C/ -1641 G haplotype has been associated with lower protein C concentrations in both homozygotes and heterozygotes as compared to -1654 T/ -1641 A (AIACH M. *et al.* Arterioscler Thromb Vasc Biol. (1999) 19(6):1573-1576).

The human endothelial protein C receptor (EPCR) gene is located on chromosome 20 and maps to chromosome 20q11.2. A representative human EPCR gene sequence with promoter is listed in GenBank under accession number AF106202 (8167 bp). A number of polymorphisms have been observed in the EPCR gene (BIGUZZI E. et al. *Thromb Haemost* (2002) 87:1085-6 and FRANCHI F. et al. *Br J Haematol* (2001) 114:641-6). Furthermore, polymorphisms of EPCR are also described in (BIGUZZI E. et al. *Thromb Haemost* (2001) 86:945-8; GALLIGAN L. et al. *Thromb Haemost* (2002) 88:163-5; ZECCHINA G. et al. *Br J Haematol* (2002) 119:881-2; FRENCH JK. et al. *Am Heart J* (2003) 145:118-24; and VON DEPKA M. et al. *Thromb Haemost* (2001) 86:1360-2; and SAPOSNIK B. et al. *Blood* (2004 Feb 15) 103(4):1311-8.).

SUMMARY OF THE INVENTION

This invention is based in part on the surprising discovery that the combination of predictive SNPs from the Protein C and EPCR can be more accurate predictors of patient outcome than SNPs from either Protein C or EPCR alone.

This invention is also based in part on the surprising discovery of protein C SNPs previously uncharacterized in the scientific literature with regards to an association with improved prognosis or patient outcome, in patients with an inflammatory condition. Furthermore, various protein C polymorphisms are provided which are useful for patient screening, as an indication of patient outcome, or for prognosis for recovery from an inflammatory condition.

This invention is also based in part on the surprising discovery that EPCR SNPs previously uncharacterized in the scientific literature with regards to an association with improved prognosis or patient outcome, in patients with an inflammatory condition. Furthermore, various protein C polymorphisms are provided which are useful for patient screening, as an indication of patient outcome, or for prognosis for recovery from an inflammatory condition.

This invention is also based in part on the surprising discovery that both EPCR and protein C SNPs alone or in combination are useful in predicting the response a patient with an inflammatory condition will have to activated protein C treatment or treatment with another anti-inflammatory agent or anti-coagulant agent.

In accordance with one aspect of the invention, methods are provided for obtaining a

prognosis or predicting ability to recover for a patient having or at risk of developing an inflammatory condition, the method comprising determining a genotype including one or more polymorphism sites in the protein C gene and/or EPCR gene for the patient, wherein said genotype is indicative of an ability of the patient to recover from an inflammatory condition. The method may further involve determination of the genotype for one or more polymorphism sites in the protein C gene and one or more polymorphism sites in the EPCR gene for the patient. The genotypes the protein C gene and/or EPCR gene may be taken alone or in combination.

The protein C polymorphism site may correspond to position 4732 of SEQ ID NO.: 1 or a polymorphism site linked thereto. Alternatively, the polymorphism site may correspond to position 4732, 4813, 6379 or 6762 of SEQ ID NO: 1. Using an alternative numbering system according to Foster et al. 4732 corresponds to position 673.

The protein C polymorphism site may correspond to position 2418 of SEQ ID NO.: 1 or a polymorphism site linked thereto. Alternatively, the polymorphism site may correspond to position 1386, 2583, 3920 or 2418 of SEQ ID NO: 1. Furthermore, the polymorphism site in linkage disequilibrium with position 2418 may correspond to a combination of two or more Protein C polymorphism sites selected from the following: 5867 and 2405; 5867 and 4919; 5867 and 4956; 5867 and 6187; 5867 and 9534; 5867 and 12109; 4800 and 2405; 4800 and 4919; 4800 and 4956; 4800 and 6187; 4800 and 9534; 4800 and 12109; 9198 and 6379 and 2405; 9198 and 6379 and 4919; 9198 and 6379 and 4956; 9198 and 6379 and 6187; 9198 and 6379 and 9534; and 9198 and 6379 and 12109.

Genotype may also be determined at a combination of two or more protein C polymorphism sites, the combination being selected from the group of positions corresponds to SEQ ID NO:1 consisting of:

9198 and 5867;
9198 and 4800;
3220 and 5867; and
3220 and 4800.

In accordance with another aspect of the invention, methods are provided for further comparing the genotype so determined with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition.

The protein C genotype of the patient may be indicative of a decreased likelihood of recovery from an inflammatory condition or indicative of a prognosis of severe cardiovascular or respiratory dysfunction in critically ill patients (risk alleles or risk genotype). Furthermore, such a genotype may be selected from the group of single polymorphism sites and combined polymorphism sites consisting of:

4732 C;
4813 A;
6379 G;
6762 A;
9198 C and 5867 A;
9198 C and 4800 G;
3220 A and 5867 A; and
3220 A and 4800 G

or

1386 T;
2418 A;
2583 A;
3920 T;
5867 A and 2405 T;
5867 A and 4919 A;
5867 A and 4956 T;
5867 A and 6187 C;
5867 A and 9534 T;
5867 A and 12109 T;
4800 G and 2405 T;
4800 G and 4919 A;

4800 G and 4956 T;
4800 G and 6187 C;
4800 G and 9534 T;
4800 G and 12109 T;
9198 A and 6379 G and 2405 T;
9198 A and 6379 G and 4919 A;
9198 A and 6379 G and 4956 T;
9198 A and 6379 G and 6187 C;
9198 A and 6379 G and 9534 T; and
9198 A and 6379 G and 12109 T..

The protein C genotype of the patient may be indicative of an increased likelihood of recovery from an inflammatory condition or indicative of a prognosis of less severe cardiovascular or respiratory dysfunction (protective alleles or non-risk genotypes) in critically ill patients. Furthermore, such a genotype may be selected from the group of single polymorphism sites and combined polymorphism sites consisting of:

4732 T;
4813 G;
6379 A;
6762 G;
9198 A and 5867 G;
9198 A and 4800 C;
3220 G and 5867 G; and
3220 G and 4800 C.

The EPCR polymorphism site may correspond to position 4054 of SEQ ID NO.: 2 or a polymorphism site linked thereto. Alternatively, the polymorphism site corresponds to position 6196, 5515, 4946, 4054, 3402, 3063 or 2973 of SEQ ID NO: 2.

In accordance with another aspect of the invention, methods are provided for further comparing the genotype so determined with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition.

The EPCR genotype of the patient may be indicative of a decreased likelihood of recovery from an inflammatory condition or indicative of a prognosis of severe cardiovascular or respiratory dysfunction in critically ill patients. Furthermore, such a genotype may be selected from the group of single polymorphism sites and combined polymorphism sites consisting of:

6196 G;
5515 T;
4946 T;
4054 T;
3402 G;
3063 G; and
2973 C.

The EPCR genotype of the patient may be indicative of an increased likelihood of recovery from an inflammatory condition or indicative of a prognosis of less severe cardiovascular or respiratory dysfunction in critically ill patients. Furthermore, such a genotype may be selected from the group of single polymorphism sites and combined polymorphism sites consisting of:

6196 C;
5515 C;
4946 C;
4054 C;
3402 C;
3063 A; and
2973 T.

In accordance with another aspect of the invention, methods are provided for identifying a polymorphism in a protein C and/or EPCR gene sequence that correlates with patient prognosis. Where the method comprises obtaining protein C and/or EPCR gene sequence information from a group of patients, identifying a site of at least one polymorphism in the

protein C and/or EPCR gene, determining genotype(s) of the site or sites for individual patients in the group, determining an ability of individual patients in the group to recover from the inflammatory condition and/or correlating genotypes determined with patient abilities and/or potential therapies.

The correlation procedure may be repeated on a patient population of sufficient size to achieve a statistically significant correlation.

The methods may further comprise steps of obtaining protein C and/or EPCR gene sequence of the patient or obtaining a nucleic acid sample from the patient. The determining of genotype may be performed on a nucleic acid sample from the patient.

Where the genotype of the patient corresponding to the nucleotide in position 4732 of SEQ ID NO: 1, is cytosine (C), the prognosis may be indicative of a decreased likelihood of recovery from an inflammatory condition or of severe cardiovascular or respiratory dysfunction in critically ill patients.

Where the genotype of the patient corresponding to the nucleotide in position 4732 of SEQ ID NO: 1, is thymine (T) the prognosis may be indicative of a increased likelihood of recovery from an inflammatory condition or of less severe cardiovascular or respiratory dysfunction in critically ill patients.

Where the genotype of the patient corresponding to the nucleotide in position 4054 of SEQ ID NO: 2, is T, the prognosis may be indicative of a decreased likelihood of recovery from an inflammatory condition or of severe cardiovascular or respiratory dysfunction in

critically ill patients.

Where the genotype of the patient corresponding to the nucleotide in position 4054 of SEQ ID NO: 2, is cytosine (C), the prognosis may be indicative of a increased likelihood of recovery from an inflammatory condition or of less severe cardiovascular or respiratory dysfunction in critically ill patients.

In accordance with another aspect of the invention, methods are provided for combining the protein C and EPCR polymorphism site genotype information to improve the predictive value for determining a patient's ability to recover from an inflammatory condition over using either a protein C or an EPCR SNP alone.

Group 1 patients have no copies of the adverse EPCR allele (4054T) and no copies of the adverse protein C allele (4732 C), group 2 patients have at least one copy of the adverse EPCR allele (4054T) and at least one copy of the adverse protein C allele or risk allele (4732C). Group 3 patients can have either at least one copy of the adverse EPCR allele or risk allele (4054T) and no copies of the adverse protein C allele (4732C) or they can have no copies of the adverse EPCR allele (4054 T) and at least one copy of the adverse protein C allele or risk allele (4732C). Group 1 patients are expected to have the best outcomes, group 2 patients are expected to have the worst outcomes and group 3 patients are expected to have intermediate outcomes.

The inflammatory condition may be selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumanitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due

to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglotittis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

The determining of a genotype may comprise one or more of: restriction fragment length analysis; sequencing; hybridization; oligonucleotide ligation assay; ligation rolling circle amplification; 5′ nuclease assay; polymerase proofreading methods; allele specific PCR; matrix assisted laser desorption ionization time of flight MALDI-TOF mass spectroscopy micro-sequencing assay; gene chip hybridization assays; and reading sequence data.

In accordance with another aspect of the invention, there is provided a kit for determining a genotype at a defined nucleotide position within a polymorphism site in a protein C gene and/or EPCR gene sequence from a patient to provide a prognosis of the patient's ability to recover from an inflammatory condition, the kit comprising, a restriction enzyme capable of distinguishing alternate nucleotides at the polymorphism site or a labeled oligonucleotide having sufficient complementary to the polymorphism site and capable of distinguishing said alternate nucleotides. The kit may also include one or more of the following: a package; instructions for using the kit to determine geneotype; reagents such a buffers, nucleotides and enzymes. A kit as described herein may contain any combination of the following: a restriction enzyme capable of distinguishing alternate nucleotides at a protein C and/or EPCR polymorphism site; and/or a labeled oligonucleotide having sufficient complementary to the protein C and/or EPCR polymorphism site and capable of distinguishing said alternate nucleotides; and/or an oligonucleotide or a set of oligonucleotides suitable for amplifying a region including the protein C and/or EPCR polymorphism site. The kit may also include one or more of the following: a package; instructions for using the kit to determine geneotype; reagents such a buffers, nucleotides and enzymes; and/or containers.

The alternate nucleotides may correspond to position 4732 of SEQ ID NO: 1, position 8 of SEQ ID NO: 3 or to a polymorphism linked thereto. The alternate nucleotides may also correspond to one or more of positions 4732, 4813 or 6379 of SEQ ID NO: 1.

The alternate nucleotides may correspond to position 6196 of SEQ ID NO: 3, position 8 of SEQ ID NO: 4 or to a polymorphism linked thereto. The alternate nucleotides may also correspond to one or more of positions 6196, 5515, 4946, 4054, 3063 or 2973 of SEQ ID NO: 2.

The kit comprising a restriction enzyme may also comprise an oligonucleotide or a set of oligonucleotides suitable to amplify a region surrounding the polymorphism site, a polymerization agent and instructions for using the kit to determine genotype.

In accordance with another aspect of the invention, there is provided a kit for determining a genotype at a defined nucleotide position within a polymorphism site in a protein C and/or EPCR gene sequence from a patient to provide a prognosis of the patient's ability to recover from an inflammatory condition, the kit comprising, in a package a restriction enzyme capable of distinguishing alternate nucleotides at the polymorphism site or a labeled oligonucleotide having sufficient complementary to the polymorphism site and capable of distinguishing said alternate nucleotides.

In accordance with another aspect of the invention, oligonucleotides are provided that may be used in the identification of protein C and/or EPCR polymorphisms in accordance with the methods described herein, the oligonucleotides are characterized in that the

oligonucleotides hybridize under normal hybridization conditions with a region of one of sequences identified by SEQ ID NO:1, SEQ ID NO:2, etc. or their complements.

In accordance with another aspect of the invention, an oligonucleotide primer is provided comprising a portion of SEQ ID NO:1, SEQ ID NO:2 or their complements, wherein said primer is twelve to fifty-four nucleotides in length and wherein the primer specifically hybridizes to a region of SEQ ID NO:1, SEQ ID NO:2 or their complements and is capable of identifying protein C and/or EPCR gene polymorphisms described herein. Alternatively, the primers may be between sixteen to twenty-four nucleotides in length.

In accordance with another aspect of the invention, methods are provided for patient screening, comprising the steps of (a) obtaining protein C and/or EPCR gene sequence information from a patient, and (b) determining the identity of one or more polymorphisms in the sequence, wherein the one or more polymorphisms may be indicative of the ability of a patient to recover from an inflammatory condition.

In accordance with another aspect of the invention methods are provided for patient screening whereby the method includes the steps of (a) selecting a patient based on risk of developing an inflammatory condition or having an inflammatory condition, (b) obtaining protein C and/or EPCR gene sequence information from the patient and (c) detecting the identity of one or more polymorphisms in the protein C gene and/or EPCR gene, wherein the polymorphism is indicative of the ability of a patient to recover from an inflammatory condition.

In accordance with another aspect of the invention, methods are provided for selecting a

group of patients to determine the efficacy of a candidate drug known or suspected of being useful for the treatment of an inflammatory condition, the method including determining a genotype for one or more polymorphism sites in the protein C gene and/or EPCR gene for each patient, wherein said genotype is indicative of the patient's ability to recover from the inflammatory condition and sorting patients based on their genotype. The method may also include administering the candidate drug to the patients or a subset of patients and determining each patient's ability to recover from the inflammatory condition. The method may also include the additional step of comparing patient response to the candidate drug based on genotype of the patient. Response to the candidate drug may be decided by determining each patient's ability to recover from the inflammatory condition.

In accordance with another aspect of the invention, methods are provided for treatment of an inflammatory condition in an eligible patient by administering a treatment option, such as a therapeutic agent, after first determining if a patient is an eligible patient on the basis of the genetic sequence information or genotype information disclosed herein. Where the method of treatment of an inflammatory condition in an eligible patient may comprise the following: a) determining if a patient is an eligible patient on the basis of the presence or absence of one or more risk genotypes in the protein C sequence and/or EPCR sequence; and b) administering a therapeutic agent to the eligible patient. More specifically, the method of treatment of an inflammatory condition in an eligible patient may comprise: a) determining if a patient is an eligible patient on the basis of the presence or absence of one or more risk genotypes in the protein C sequence and/or EPCR sequence; and b) administering a therapeutic agent selected from among activated protein C (e.g. XIGRIS™ - drotecogin alfa-recombinant human activated protein C (Eli Lilly)), tissue factor pathway inhibitors (e.g. TIFACOGIN™ - alpha (Chiron) and the like), platelet activating factor hydrolase (e.g. PAFase™ (ICOS) and other PAF-AH enzyme analogues), antibody to tumor necrosis factor- alpha (e.g. SEGARD™ - afelimomab (Abbott)), or other anti-inflammatory therapeutic agent, to the eligible patient. Furthermore, the therapeutic agent may be activated protein C and/or a derivative thereof (including glycosylation mutants), alone or in combination or in combination with other therapeutic agents as described herein. An improved response to a therapeutic agent may include an improvement

subsequent to administration of the therapeutic agent, whereby the patient has an increased likelihood of survival, reduced likelihood of organ damage or organ dysfunction (Brussels score), an improved APACHE II score, days alive and free of pressors, inotropes, and reduced systemic dysfunction (cardiovascular, respiratory, ventilation, CNS, coagulation [INR> 1.5], renal and/or hepatic).

In accordance with another aspect of the invention, methods are provided for treatment of an inflammatory condition in an eligible patient comprising administering a therapeutic agent to an eligible patient. The eligible patient may be a patient having one or more of the polymorphisms in protein C and/or EPCR that are associated with decreased likelihood of recovery from an inflammatory condition, as disclosed herein or as later discovered. Treatment options, may include: activated protein C (e.g. XIGRIS™ drotecogin alfa-recombinant human activated protein C (Eli Lilly)), tissue factor pathway inhibitors (e.g. TIFACOGIN™ alpha (Chiron) and the like), platelet activating factor hydrolase (e.g. PAFase™ (ICOS) and other PAF-AH enzyme analogues), antibody to tumor necrosis factor- alpha (e.g. SEGARD™ afelimomab (Abbott)), soluble tumor necrosis factor receptor-immuoglobulin G1 (Roche), procysteine, elastase inhibitor, human recombinant interleukin 1 receptor antagonist (IL-1 RA), and antibodies, inhibitors and antagonists to: an endotoxin (i.e. lipopolysaccharide, LPS, lipotechoic acid and the like, e.g. E-5531 (Eisai)), tumour necrosis factor receptor, IL-6, high-mobility group box 1 (HMGB-1 or HMG-1), tissue plasminogen activator, bradykinin, CD-14, and/or IL-10. Those skilled in the art are familiar with the dosage and administration of these and other treatment options. To determine a patient's eligibility, the presence or absence of polymorphisms in the protein C sequence and/or EPCR sequence, may be determined as described herein.

Activated protein C (e.g. XIGRIS™ drotecogin alfa-recombinant human activated protein C (Eli Lilly)), tissue factor pathway inhibitors (e.g. TIFACOGIN™ alpha (Chiron) and the like), platelet activating factor hydrolase (e.g. PAFase™ (ICOS) and other PAF-AH enzyme analogues), antibody to tumor necrosis factor- alpha (e.g. SEGARD™ afelimomab (Abbott)), or other anti-inflammatory therapeutic agent, may be useful in the manufacture of a medicament for the therapeutic treatment of an inflammatory condition in a patient having one or more of the polymorphisms in protein C and/or EPCR that are

associated with decreased likelihood of recovery from an inflammatory condition. Furthermore these therapeutic agents may be useful in the preparation of an anti-sepsis agent in ready-to-use drug form for treating or preventing sepsis in a patient having one or more of the polymorphisms in protein C and/or EPCR that are associated with decreased likelihood of recovery from an inflammatory condition.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient in need thereof, the method including administering to the patient an anti-inflammatory agent or an anti-coagulant agent, wherein said patient has a protein C gene or EPCR gene risk genotype.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient in need thereof, the method including selecting a patient having a risk genotype in their protein C gene or EPCR gene and administering to the patient an anti-inflammatory agent or an anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a mammal in need thereof, the method including administering to the mammal an anti-inflammatory agent or an anti-coagulant agent, wherein the mammal has a protein C gene or EPCR gene risk genotype.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a mammal in need thereof, the method including selecting a mammal having a risk genotype in their protein C gene or EPCR gene and administering to the mammal an anti-inflammatory agent or an anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, including the step of identifying a subject having one or more risk genotypes in their protein C gene or EPCR gene, wherein the identification of a subject with one or more risk genotypes is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for treating a subject with an inflammatory condition by administering an anti-inflammatory agent or an anti-coagulant agent, the method including administering the anti-inflammatory agent or the anti-coagulant agent to subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for identifying a subject with increased responsiveness to treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, including the step of screening a population of subjects to identify those subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with a risk genotype in their protein C gene or EPCR gene is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, including the step of identifying a subject having a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient, the method including administering an anti-inflammatory agent or an anti-coagulant agent to the patient, wherein said patient has a risk genotype in their protein C gene or EPCR gene.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient, the method including identifying a patient having a

risk genotype in their protein C gene or EPCR gene and administering an anti-inflammatory agent or an anti-coagulant agent to the patient.

In accordance with another aspect of the invention, the use of an anti-inflammatory agent or an anti-coagulant in the manufacture of a medicament are provided for the treatment of an inflammatory condition, wherein the patients treated have a risk genotype in their protein C gene or EPCR gene.

In accordance with another aspect of the invention, methods may further comprise determining the patients APACHE II score as an assessment of patient risk and/or determining the number of organ system failures for the patient as an assessment of patient risk. A patient having an APACHE II score when ≥ 25 and/or having 2 or more organ system failures may be indicative of increased risk.

Risk geneotypes may be selected alone or in combination from the following protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1:

4732 C;
4813 A;
6379 G;
6762 A;
9198 C and 5867 A;
9198 C and 4800 G;
3220 A and 5867 A; and
3220 A and 4800 G

or

1386 T;
2418 A;
2583 A;
3920 T;
5867 A and 2405 T;
5867 A and 4919 A;
5867 A and 4956 T;
5867 A and 6187 C;
5867 A and 9534 T;

5867 A and 12109 T;
4800 G and 2405 T;
4800 G and 4919 A;
4800 G and 4956 T;
4800 G and 6187 C;
4800 G and 9534 T;
4800 G and 12109 T;
9198 A and 6379 G and 2405 T;
9198 A and 6379 G and 4919 A;
9198 A and 6379 G and 4956 T;
9198 A and 6379 G and 6187 C;
9198 A and 6379 G and 9534 T; and
9198 A and 6379 G and 12109 T.

Risk geneotypes may be selected alone or in combination from the following EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

6196 G;
5515 T;
4946 T;
4054 T;
3402 G;
3063 G; and
2973 C.

Risk genotype may be an indication of an increased risk of not recovering from an inflammatory condition and may also indicative of an improved likelihood of responding favourable to an anti-inflammatory agent or the anti-coagulant agent. An anti-inflammatory agent or the anti-coagulant agent may be selected from the following activated protein C; tissue factor pathway inhibitors; platelet activating factor hydrolase; PAF-AH enzyme analogues; antibody to tumor necrosis factor alpha; soluble tumor necrosis factor receptor-immunoglobulin G1; procysteine; elastase inhibitor; human recombinant interlukin 1 receptor antagonists; and antibodies, inhibitors and antagonists to endotoxin, tumour necrosis factor receptor, interleukin-6, high mobility group box, tissue plasminogen activator, bradykinin, CD-14 and interleukin-10. An anti-inflammatory

agent or the anti-coagulant agent is preferebly an activated protein C (such as drotecogin alfa activated or XIGRIS™) or derivative or analog thereof.

Non-risk geneotypes or protective genotypes may be selected alone or in combination from the following protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1:

4732 T;
4813 G;
6379 A;
6762 G;
9198 A and 5867 G;
9198 A and 4800 C;
3220 G and 5867 G; and
3220 G and 4800 C

or

1386 C;
2418 G;
2583 T;
3920 C;
5867 G and 2405 C;
5867 G and 4919 G;
5867 G and 4956 C;
5867 G and 6187 T;
5867 G and 9534 C;
5867 G and 12109 C;
4800 C and 2405 C;
4800 C and 4919 G;
4800 C and 4956 C;
4800 C and 6187 T;
4800 C and 9534 C; and
4800 C and 12109 C.

Non-risk geneotypes or protective genotypes may be selected alone or in combination from the following EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

6196 C;
5515 C;
4946 C;

4054 C;
3402 C;
3063 A; and
2973 T.

The above identified sequence positions refer to the sense strand of the protein C gene and/or EPCR gene as indicated. It will be obvious to a person skilled in the art that analysis could be conducted on the anti-sense strand to determine patient outcome.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows shows haplotypes and haplotype clades of the protein C gene.

FIG. 2 shows haplotypes and haplotype clades of the endothelial cell protein C (EPCR) receptor gene.

FIG. 3 shows phylogenetic tree of EPCR haplotypes generated with MEGA2 software.

FIG. 4 shows Days Alive and Free of Acute Lung Injury/ARDS by EPCR Haplotype Clade.

FIG. 5 shows a Kaplan-Meier curve of the survival of groups 1, 2 and 3 of the protein C/EPCR haplotypes over 28 days.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

In the description that follows, a number of terms are used extensively, the following definitions are provided to facilitate understanding of the invention.

"Genetic material" includes any nucleic acid and can be a deoxyribonucleotide or ribonucleotide polymer in either single or double-stranded form.

A "purine" is a heterocyclic organic compound containing fused pyrimidine and imidazole rings, and acts as the parent compound for purine bases, adenine (A) and guanine (G). "Nucleotides" are generally a purine (R) or pyrimidine (Y) base covalently linked to a pentose, usually ribose or deoxyribose, where the sugar carries one or more phosphate groups. Nucleic acids are generally a polymer of nucleotides joined by 3′ 5′ phosphodiester linkages. As used herein "purine" is used to refer to the purine bases, A and G, and more broadly to include the nucleotide monomers, deoxyadenosine-5′ -phosphate and deoxyguanosine-5′ -phosphate, as components of a polynucleotide chain.

A "pyrimidine" is a single-ringed, organic base that forms nucleotide bases, cytosine (C), thymine (T) and uracil (U). As used herein "pyrimidine" is used to refer to the pyrimidine bases, C, T and U, and more broadly to include the pyrimidine nucleotide monomers that along with purine nucleotides are the components of a polynucleotide chain.

A nucleotide represented by the symbol M may be either an A or C, a nucleotide represented by the symbol W may be either an T or A, a nucleotide represented by the symbol S may be either an G or C, while a nucleotide represented by the symbol R may be either an G or A.

A "polymorphic site" or "polymorphism site" or "polymorphism" or "single nucleotide polymorphism site" (SNP site) as used herein is the locus or position with in a given sequence at which divergence occurs. A "Polymorphism" is the occurrence of two or

more forms of a gene or position within a gene (allele), in a population, in such frequencies that the presence of the rarest of the forms cannot be explained by mutation alone. The implication is that polymorphic alleles confer some selective advantage on the host. Preferred polymorphic sites have at least two alleles, each occurring at frequency of greater than 1%, and more preferably greater than 10% or 20% of a selected population. Polymorphism sites may be at known positions within a nucleic acid sequence or may be determined to exist using the methods described herein. Polymorphisms may occur in both the coding regions and the noncoding regions (for example, promoters and introns) of genes.

A "risk genotype" as used herein refers an allelic variant (genotype) at one or more polymorphism sites within the Protein C gene or EPCR gene described herein as being indicative of a decreased likelihood of recovery from an inflammatory condition or an increased risk of having a poor outcome. The risk geneotype may be determined for either the haploid genotype or diploid geneotype, provided that at least one copy of a risk allele is present. Such "risk alleles" or "risk genotype" may be selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 C;
4813 A;
6379 G;
6762 A;
9198 C and 5867 A;
9198 C and 4800 G;
3220 A and 5867 A; and
3220 A and 4800 G

or

1386 T;
2418 A;

2583 A;
3920 T;
5867 A and 2405 T;
5867 A and 4919 A;
5867 A and 4956 T;
5867 A and 6187 C;
5867 A and 9534 T;
5867 A and 12109 T;
4800 G and 2405 T;
4800 G and 4919 A;
4800 G and 4956 T;
4800 G and 6187 C;
4800 G and 9534 T;
4800 G and 12109 T;
9198 A and 6379 G and 2405 T;
9198 A and 6379 G and 4919 A;
9198 A and 6379 G and 4956 T;
9198 A and 6379 G and 6187 C;
9198 A and 6379 G and 9534 T; and
9198 A and 6379 G and 12109 T.

Or such "risk alleles" or "risk genotype" may be selected from the group of EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

6196 G;
5515 T;
4946 T;
4054 T;
3402 G;
3063 G; and
2973 C.

Furthermore, protein C (4732; 2418 and single or combined polymorphism sites in total linkage disequilibrium or having a high degree of linkage disequilibrium $D' \geq 0.8$) and EPCR 4054 and single or combined polymorphism sites in total linkage disequilibrium or having a high degree of linkage disequilibrium ($D' \geq 0.8$) "risk alleles" or "risk genotypes" may be combined to improve the predictive value and to improve the determinative value in deciding whether to treat a patient or mammal with an anti-inflammatory agent or an anti-coagulant agent.

A "clade" is a group of haplotypes that are closely related phylogenetically. For example, if haplotypes are displayed on a phylogenetic (evolutionary) tree a clade includes all haplotypes contained within the same branch.

As used herein "haplotype" is a set of alleles of closely linked loci on a chromosome that tend to be inherited together; commonly used in reference to the linked genes of the major histocompatibility complex.

As used herein "linkage disequilibrium" is the occurrence in a population of certain combinations of linked alleles in greater proportion than expected from the allele frequencies at the loci. Accordingly, if the geneotype of a first locus corressponds to a second locus (or third locus etc.), the identification of only one locus would necessarily provide the identity of the other locus, provided the loci were in "total linkage disequilibrium" or have a high degree of linkage disequilibrium (i.e. an absolute value for D' of ≥ 0.8 or $r^2 \geq 0.8$). Alternatively, a high degree of linkage disequilibrium may be represented by an absolute value for D' of ≥ 0.85 or $r^2 \geq 0.85$ or by an absolute value for D' of ≥ 0.9 or $r^2 \geq 0.9$. The terms "complete linkage disequilibrium" or "total linkage disequilibrium" are used to refer to a population association between two loci that are linked wherein the two or more loci (SNPs) always have the same corresponding geneotypes. However, two SNPs that have a high degree of linkage disequilibrium (i.e. LD' ≥ 0.8) are also useful in determining the genotype of a corresponding SNP. Therefore, we assume that a tested SNP is representative of another SNP in "total linkage disequilibrium" or having a high degree of linkage disequilibrium. For example, in the population from which the haplotype map was created the SNP at position 4054 of SEQ. ID NO.: 2 was in "total linkage disequilibrium" with position 6196 of SEQ. ID NO.: 2, whereby when the genotype of 4054 is T the genotype of 6196 is G. Similarly, when the genotype of 4054 is C the genotype of 6196 is C. Accordingly, the determination of the genotype of a single locus will necessarily provide the identity of the genotype of any locus in "total linkage disequilibrium" therewith and is likely to provide the identity of the genotype of any locus having a high degree of linkage disequilibrium thereto.

The "promoter" region is 5′ or upstream of the translation start site, in this case the translation start site is located at position 4062 of TABLE 1A (SEQ. ID NO: 1) and the transcription start site is located at position 2559 of TABLE 1A (SEQ. ID NO: 1).

Numerous sites have been identified as polymorphism sites in the EPCR gene, where those polymorphisms are linked to the polymorphism at position 4054 of SEQ. ID NO: 2 and may also therefore be indicative of patient prognosis. The following single polymorphism sites are linked to 4054 of SEQ. ID NO.: 2:

6196;
5515;
4946;
3402;
3063; and
2973.

It will be appreciated by a person of skill in the art that further linked single polymorphism sites and combined polymorphism sites could be determined. The haplotype of protein C or EPCR can be created by assessing the SNPs of protein C and/or EPCR in normal subjects using a program that has an expectation maximization algorithm (i.e. PHASE). A constructed haplotype of protein C and/or EPCR may be used to find combinations of SNP's that are in total linkage disequilibrium (LD) with 4732 of SEQ ID NO: 1 and/or 4054 of SEQ ID NO: 2. Therefore, the haplotype of an individual could be determined by genotyping other SNPs that are in total LD with 4732 of SEQ ID NO: 1 and/or 4054 of SEQ ID NO: 2. Linked single polymorphism sites or combined polymorphism sites may also be genotyped for assessing patient prognosis.

Previously identified single nucleotide polymorphisms in the protein C gene were described in international patent application, PCT/CA03/00751, which is incorporated herein by reference. Polymorphism sites in the protein C gene previosly identified correspond to position 2418 of SEQ ID NO.: 1 or polymorphism sites in total linkage disequilibrium thereto. Such polymorphism may also be used as risk genotypes alone or in combination with other Protein C or EPCR gene risk genotypes in determining a patient's suitability for administration the anti-inflammatory agent or the anti-coagulant agent.

The following genotypes for single polymorphism sites and combined polymorphism sites in SEQ ID NO: 2 may indicative of a decreased likelihood of recovery from an inflammatory condition or indicative of severe cardiovascular or respiratory dysfunction in critically ill patients (risk alleles):

6196 G;
5515 T;
4946 T;
4054 T;
3402 G;
3063 G; and
2973 C.

Whereas the following genotypes for single polymorphism sites and combined polymorphism sites in SEQ ID NO: 2 may indicative of a increased likelihood of recovery from an inflammatory condition or indicative of less severe cardiovascular or respiratory dysfunction in critically ill patients (protective alleles):

6196 C;
5515 C;
4946 C;
4054 C;
3402 C;
3063 A; and
2973 T.

It will be appreciated by a person of skill in the art, that the numerical designations of the positions of polymorphisms within a sequence are relative to the specific sequence. Also the same positions may be assigned different numerical designations depending on the way in which the sequence is numbered and the sequence chosen, as illustrated by the alternative numbering of equivalent polymorphisms in Foster *et al.* and Millar *et al.* above. Furthermore, sequence variations within the population, such as insertions or deletions, may change the relative position and subsequently the numerical designations of particular nucleotides at and around a polymorphism site.

TABLE 1A below is representative of a *Homo sapiens* protein C gene sequence and comprises a sequence as listed in GenBank under accession number AF378903. The SNPs described as -1654 C/T, -1641 A/G and -1476 A/T using the numbering system of Foster *et al.* correspond to 2405, 2418 and 2583 respectively in TABLE 1A (SEQ ID NO:1. Polymorphism sites shown below in TABLE 1A are shown in bold and are capitalized. The major and minor alleles for each of the 4732 and linked polymorphism sites of the protein C gene are as follows (combination sites are not given here):

at position 4732 the most common nucleotide (major allele) is **t** and the minor allele is **c**;

at position 4813 the most common nucleotide (major allele) is **g** and the minor allele is **a**;

at position 6379 the most common nucleotide (major allele) is **a** and the minor allele is **g**;

at position 9198 the most common nucleotide (major allele) is **a** and the minor allele is **c**;

at position 5867 the most common nucleotide (major allele) is **g** and the minor allele is **a**;

at position 4800 the most common nucleotide (major allele) is **c** and the minor allele is **g**;

at position 3220 the most common nucleotide (major allele) is **g** and the minor allele is **a**.

TABLE 1A

```
1     gctctctaac tcacagcgag ctcgctgccc aaagtcctgc tccgggggct tcctgggtgg
61    acctgaccgc gttcgggtgc acgtggggcg actcacacct gacaagtaaa gcgggtgagg
121   ccgcgcctgt gaagggcgcc tggctcctcc gcaggacggt gcggcgcggc gccccggct
181   ggaaccaggt gtaactgcag agaccctggg atcgcaggaa cggctggcgg caggactgtc
241   cctacctcga gaaggtgacg gggtttcctg cgctgccagc cgatgaggcg gccgtgacgc
301   agcccgccgt gcagagtccc cgtcggccga caggcgtgca gagctctgca gaggaccctt
361   ccgccctctg ggcagcctgc caagccgtgg caccccccaac ccccagcact gggcacttgg
421   gagcattgca gccgccctgg ctcgtaccgg tgccggtgct ttgggcacct gggctggttt
481   ggacatgggt gccccgggca gagtccattt atgcaggtca gaatcagtgt gtggagcctg
541   catagacttg ccctggagcg gctgcctgtg ctggggtggg gaggagtaga gggcgagaag
601   ttggtgggga agggaagcgg cgccaaaaga atacccacaa catcttgcac ctggaaggca
661   aagcagaggg cagtgatctc tgcagacttg cgggggcgac gcctgaagca aacagggaca
721   tacaagctgg tgccttctgt ggttgtgcat ggggtcttca tgcttcctgt ctgagttccc
781   agaagcttgt ctctgctttt ctaggcagct gccacagcct gtcacaaaca gctcctggtt
841   ctccacttct catagtctcg atttcaaaat ccattgcctc accctccacc tcctctccac
901   ctccacccct cctagcacct cctgactgct tgtgttctgt gtctccccac tgtctcccaa
961   cctggggtgg ggttgggggg gatgtctttc ctcctgtctg ctctttgatg tccagctgaa
1021  gtgtcacctc ctacaggcag cctcccctgg ctatgccagc ttgtactgat tgccctctcc
1081  tctgaattct gtaagcattt cctatgtgta cctgcccctg ggcaaggtgg gcctgacttg
1141  ttagagtgtt agagttttac cctgttcctc taggagggcc tggtaccacc acagcccagc
1201  atggtgtggt gcctcagcag gaggcatctg gttacaatca acacaagctg ttccagccaa
1261  tttaaagaaa cttcaggagg aatagggttt taggagggca tggggaccct cctgcacccg
1321  aagccaggat gtgccaccaa tcataaggag gcaggggcct ccttccgctg ctccctggga
1381  ctctcYaggt gtccgtggcc tcagcccccc tctgcacacc tgcatcttcc ttctcatcag
1441  cttcctctgc tttaagcgta aacatggatg cccaggacct ggcctcaatc ttccgagtct
1501  ggtacttatg gtgtactgac agtgtgagac cctactcctc tgatcaatcc cctgggttgg
1561  tgacttccct gtgcaatcaa tggaagccag cgaggcaggg tcacatgccc cgtttagagg
1621  tgcagacttg gagaaggaac gtgggcaagt cttcccagga acaggtaggg cagggaggaa
1681  agggggggcat ctctggtgca gcccggttcg gagcaggaag acgcttaata aatgctgata
1741  gactgcagga cacaggcaaa ggtgctgagc tggacccttt atttctgccc ttctcccttc
1801  tggcaccccg gccaggaaat tgctgcagcc tttctggaat cccgttcatt tttcttactg
1861  gtccacaaaa ggggccaaat ggaagcagca agacctgagt tcaaattaaa tctgccaact
1921  accagctcag tgaatctggg cgagtaacac aaaacttgag tgtccttacc tgaaaaatag
1981  aggttagagg gatgctatgt gccattgtgt gtgtgtgttg ggggtgggga ttgggggtga
2041  tttgtgagca attggaggtg agggtggagc ccagtgccca gcacctatgc actggggacc
2101  caaaaaggag catcttctca tgattttatg tatcagaaat tgggatggca tgtcattggg
2161  acagcgtctt ttttcttgta tggtggcaca taaatacatg tgtcttataa ttaatggtat
```

2221 tttagatttg acgaaatatg gaatattacc tgttgtgctg atcttgggca aactataata
2281 tctctgggca aaaatgtccc catctgaaaa acagggacaa cgttcctccc tcagccagcc
2341 actatggggc taaaatgaga ccacatctgt caagggtttt gccctcacct ccctccctgc
2401 tggaYggcat ccttggtRgg cagaggtggg cttcgggcag aacaagccgt gctgagctag
2461 gaccaggagt gctagtgcca ctgtttgtct atggagaggg aggcctcagt gctgagggcc
2521 aagcaaatat ttgtggttat ggattaactc gaactccagg ctgtcatggc ggcaggacgg
2581 cgWacttgca gtatctccac gacccgcccc tgtgagtccc cctccaggca ggtctatgag
2641 gggtgtggag ggagggctgc ccccgggaga agagagctag gtggtgatga gggctgaatc
2701 ctccagccag ggtgctcaac aagcctgagc ttggggtaaa aggacacaag gccctccaca
2761 ggccaggcct ggcagccaca gtctcaggtc cctttgccat gcgcctccct ctttccaggc
2821 caagggtccc cagggcccag ggccattcca acagacagtt tggagcccag gaccctccat
2881 tctccccacc ccacttccac ctttgggggt gtcggatttg aacaaatctc agaagcggcc
2941 tcagagggag tcggcaagaa tggagagcag ggtccggtag ggtgtgcaga gggccacgtg
3001 gcctatccac tggggagggt tccttgatct ctggccacca gggctatctc tgtggccttt
3061 tggagcacct ggtggtttgg ggcaggggtt gaatttccag gcctaaaacc acacaggcct
3121 ggccttgagt cctggctctg cgagtaatgc atggatgtaa acatggagac ccaggacctt
3181 gcctcagtct tccgagtctR gtgcctgcag tgtactgatg gtgtgagacc ctactcctgg
3241 aggatggggg acagaatctg atcgatcccc tgggttggtg acttccctgt gcaatcaacg
3301 gagaccagca agggttggat ttttaataaa ccacttaact cctccgagtc tcagtttccc
3361 cctctatgaa atggggttga cagcattaat aactacctct tgggtggttg tgagccttaa
3421 ctgaagtcat aatatctcat gtttactgag catgagctat gtgcaaagcc tgttttgaga
3481 gctttatgtg gactaactcc tttaattctc acaacaccct ttaaggcaca gatacaccac
3541 gttattccat ccattttaca aatgaggaaa ctgaggcatg gagcagttaa gcatcttgcc
3601 caacattgcc ctccagtaag tgctggagct ggaatttgca ccgtgcagtc tggcttcatg
3661 gcctgccctg tgaatcctgt aaaaattgtt tgaaagacac catgagtgtc caatcaacgt
3721 tagctaatat tctcagccca gtcatcagac cggcagaggc agccacccca ctgtccccag
3781 ggaggacaca aacatcctgg caccctctcc actgcattct ggagctgctt tctaggcagg
3841 cagtgtgagc tcagccccac gtagagcggg cagccgaggc cttctgaggc tatgtctcta
3901 gcgaacaagg accctcaatY ccagcttccg ccctgacggc cagcacacag ggacagccct
3961 ttcattccgc ttccacctgg gggtgcaggc agagcagcag cgggggtagg cactgcccgg
4021 agctcagaag tcctcctcag acaggtgcca gtgcctccag aatgtggcag ctcacaagcc
4081 tcctgctgtt cgtggccacc tggggaattt ccggcacacc agctcctctt ggtaaggcca
4141 ccccacccct accccgggac ccttgtggcc tctacaaggc ctggtggcat ctgcccaggc
4201 cttcacagct tccaccatct ctctgagccc tgggtgaggt gaggggcaga tgggaatggc
4261 aggaatcaac tgacaagtcc caggtaggcc agctgccaga gtgccacaca ggggctgcca
4321 gggcaggcat gcgtgatggc agggagcccc gcgatgacct cctaaagctc cctcctccac
4381 acggggatgg tcacagagtc ccctgggcct tccctctcca cccactcact ccctcaactg
4441 tgaagacccc aggcccaggc taccgtccac actatccagc acagcctccc ctactcaaat
4501 gcacactggc ctcacggctg ccctgcccca acccctttcc tggtctccac agccaacggg
4561 aggaggccat gattcttggg gaggtccgca ggacacatgg gcccctaaag ccacaccagg

4621 ctgttggttt catttgtgcc tttatagagc tgtttatctg cttgggacct gcacctccac
4681 cctttcccaa ggtgccctca gctcaggcat accctcctct aggatgcctt tYcccccatc
4741 ccttcttgct cacaccccca acttgatctc tccctcctaa ctgtgccctg cacccaagaS
4801 agacacttca caRagcccag gagacacctg gggacccttc ctgggtgata ggtctgtcta
4861 tcctccaggt gtccctgccc aaggggagaa gcatggggaa tacttggttg ggggaggaRa
4921 ggaagactgg ggggatgtgt caagatgggg ctgcaYgtgg tgtactggca gaagagtgag
4981 aggatttaac ttggcagcct ttacagcagc agccagggct tgagtactta tctctgggcc
5041 agggactgta ttggatgttt tacatgacgg tctcatcccc atgtttttgg atgagtaaat
5101 tgaaccttag aaaggtaaag acactggctc aaggtcacac agagatcggg gtggggttca
5161 cagggaggcc tgtccatctc agagcaaggc ttcgtcctcc aactgccatc tgcttcctgg
5221 ggaggaaaag agcagaggac ccctgcgcca agccatgacc tagaattaga atgagtcttg
5281 agggggcgga gacaagacct tcccaggctc tcccagctct gcttcctcag acccccctcat
5341 ggccccagcc cctcttaggc ccctccacca aggtgagctc cccctccctc caaaaccaga
5401 ctcagtgttc tccagcagcg agcgtgccca ccaggtgctg cggatccgca aacgtgccaa
5461 ctccttcctg gaggagctcc gtcacagcag cctggagcgg gagtgcatag aggagatctg
5521 tgacttcgag gaggccaagg aaattttcca aaatgtggat gacacagtaa ggccaccatg
5581 ggtccagagg atgaggctca ggggcgagct ggtaaccagc aggggcctcg aggagcaggt
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5761 agctccagga aagagtgtgg ggtgacccta ggtggggact cccaccagcc acagtgtagg
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6121 gtaacggggc tggcttcctg agacaaggct cagacccgct ctgtccctgg ggatcgcttc
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11521 agtattttgc agtagggggt tctctggtgc cctcttcgaa tctgggcaca ggtacctgca
11581 cacacatgtt tgtgaggggc tacacagacc ttcacctctc cactcccact catgaggagc
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11881 gaaaggccaa attcacattt cctacagcat aatctatggc cagtggcccc ccgtggggct
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12421 actggggaga ggctccccgc agcccactct gactgtgccc tctgccctgc aggagagtat
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12721 gagaaggagg ccaagagaaa ccgcaccttc gtcctcaact tcatcaagat tcccgtggtc
12781 ccgcacaatg agtgcagcga ggtcatgagc aacatggtgt ctgagaacat gctgtgtgcg
12841 ggcatcctcg gggaccggca ggatgcctgc gagggcgaca gtgggggggcc catggtcgcc
12901 tccttccacg gcacctggtt cctggtgggc ctggtgagct ggggtgaggg ctgtgggctc
12961 cttcacaact acggcgttta caccaaagtc agccgctacc tcgactggat ccatgggcac
13021 atcagagaca aggaagcccc ccagaagagc tgggcacctt agcgaccctc cctgcagggc
13081 tgggcttttg catggcaatg gatgggacat taaagggaca tgtaacaagc acaccggcct
13141 gctgttctgt ccttccatcc ctcttttggg ctcttctgga gggaagtaac atttactgag
13201 cacctgttgt atgtcacatg ccttatgaat agaatcttaa ctcctagagc aactctgtgg
13261 ggtggggagg agcagatcca agttttgcgg ggtctaaagc tgtgtgtgtt gaggggggata
13321 ctctgtttat gaaaaagaat aaaaaacaca accacgaagc cactagagcc ttttccaggg
13381 ctttgggaag agcctgtgca agccggggat gctgaaggtg aggcttgacc agctttccag
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13741 cagtaagaaa gaggctctgg cttaacctgg cctgataggc ctaattgaat gagacagaaa
13801 taagtcaagg atgctctgat ttgaaatcat gaagtacctg atgaaaagaa atggtggtga
13861 gataaagctg

TABLE 1B below is representative of a Human endthelial cell protein C receptor (EPCR) gene sequence (SEQ ID NO:2). Polymorphism sites shown below in TABLE 1B are shown in bold and capitalized. The major and minor alleles for each of the primary polymorphism sites of the EPCR gene are as follows:

at position 6196 the most common nucleotide (major allele) is **g** and the minor allele is **c**;

at position 5515 the most common nucleotide (major allele) is **t** and the minor allele is **c**;

at position 4946 the most common nucleotide (major allele) is **t** and the minor allele is **c**;

at position 4054 the most common nucleotide (major allele) is **t** and the minor allele is **c**;

at position 3402 the most common nucleotide (major allele) is **g** and the minor allele is **c**;

at position 3063 the most common nucleotide (major allele) is **g** and the minor allele is **a**;

at position 2973 the most common nucleotide (major allele) is **c** and the minor allele is **t**.

TABLE 1B

tagagaagcg agaccacatc tctagtaaaa ataaaaaaaa aatagctagg 50
cgtggtggca cagtggcacg tacctttagt ctcagctact cgggtggttg 100
aggtgggaga atcacttgag cccgggaggt caagcctaca attagctgtg 150
attgcttcac tgcactatag cctgggcaac agagctagac cctgtctcaa 200
aaaaataata ataaatttta tatatatata tgaggatgaa attacatatg 250
tattatttga acagaagtga aatcttttct ttttttttt caaaaaaaat 300
tttgccgcat gccccaggct aaaatgcagt ggtgtgatct gggccctctg 350
aaacctccac ctcccgggtt caagggattc tcatgcctcg gtctcccaag 400
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ttcgtagaga cgttcgccat attggccagg ctggtctcaa actcctggcc 500
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ttcatagatc tccgtccttc ccttcccaca gcccccagca cttcaccccc 4700
caccctccag ccacttctca tacaagctga tgacttcgct cttagctcca 4750
ctcatgaccc gaactcttcc cccaaagacc ccaagttctt ctctcaaagc 4800

cccactcctt ccccgtcaca accctaactc cttcttctca aagaccccaa 4850
tttctttttct caaagcacca agcaccactc cgtccccctt cccccaccat 4900
catggccttt aactcctttc tctcctagtc ccccacccca cccccYtttt 4950
ttttttttttt ttttttttttt gagacggagt cttgctctgt cgtccaggct 5000
ggagtgcagt ggcgcgatct cggctcactg caacttccgc ctcccgggtt 5050
caagcgattc tcctgcctca gcctcccaag cagctgggac tacaggcacc 5100
cgccaccacg cccggctaat tttttgtatt tttagtagag acggggtttc 5150
gccatgttgg ccaggctggt ctcgaactcc tgacctcagg cgatccacaa 5200
gcctggcctc ccaaagtgct gggattacag gcgtgagctg ccgcccctgc 5250
cccagcctca ccccctgttt tttttttcta ttacagttga acaaggcctg 5300
acaattccct tttttcatca cagtccctgg ccccttcttt cttagcctct 5350
aacaggctaa ccccaaaccc ctcctcacag ccccaggccc ttctccccat 5400
agttccctga cctagactcc cctctcctca cagcactgac tcttgccttc 5450
tcatgttctt ttccccttgg tgggcctcgc ccacacctgg caccctctct 5500
gcacagtccc ctgaYcctga ctgtctatcc acagttcctc tgaccatccg 5550
ctgcttcctg ggctgtgagc tgcctcccga gggctctaga gcccatgtct 5600
tcttcgaagt ggctgtgaat gggagctcct ttgtgagttt ccggccggag 5650
agagccttgt ggcaggcaga cacccaggtc acctccggag tggtcacctt 5700
caccctgcag cagctcaatg cctacaaccg cactcggtat gaactgcggg 5750
aattcctgga ggacacctgt gtgcagtatg tgcagaaaca tatttccgcg 5800
gaaaacacga aaggtatgat gggacggggc ccaggcctgc aagctgggga 5850
gagggcgggt tccagacaaa tggatggacc tgaaggatgg atgcctagag 5900
caacaagagg cccacagctg ggggtttggg acagaacaca cgcagcttca 5950
gtcagttggt aaacgggtcc ctttcctctg gggcagaaac gctttggggt 6000
ttgactcaaa tcatggactc cttgggggcc tattcttcgg gctaactctt 6050
tgcatgttct gcagggagcc aaacaagccg ctcctacact tcgctggtcc 6100
tgggcgtcct ggtgggcagt ttcatcattg ctggtgtggc tgtaggcatc 6150
ttcctgtgca caggtggacg gcgatgttaa ttactctcca gccccStcag 6200
aaggggctgg attgatggag gctggcaagg gaaagtttca gctcactgtg 6250
aagccagact ccccaactga aacaccagaa ggtttggagt gacagctcct 6300
ttcttctccc acatctgccc actgaagatt tgagggaggg gagatggaga 6350
ggagaggtgg acaaagtact tggtttgcta agaacctaag aacgtgtatg 6400
ctttgctgaa ttagtctgat aagtgaatgt ttatctatct ttgtggaaaa 6450
cagataatgg agttggggca ggaagcctat ggcccatcct ccaaagacag 6500
acagaatcac ctgaggcgtt caaaagatat aaccaaataa acaagtcatc 6550
cacaatcaaa atacaacatt caatacttcc aggtgtgtca gacttgggat 6600
gggacgctga tataataggg tagaaagaag taacacgaag aagtggtgga 6650
aatgtaaaat ccaagtcata tggcagtgat caattattaa tcaattaata 6700
atattaataa atttcttata tttaaggcat tgttatctcc tccactttgc 6750
aaaatttctg gaaaagtaac ctatacccat ttcttctgct tccttatttc 6800
tcactcattc tttttttttt tttttttttt tttgagacag agtcttgctc 6850
tgttgcctag gctggagtgc aatggtgtga tctcagctca ctgcaacctc 6900
tgcctcccgg ttcaagcaat tctcctgcct cagcctccca agcagctggg 6950
attacagatg catgccacca cacccagcta atttttgtat ttttagtaga 7000
gatggggttt caccacgttg gccatcctga cctcgtgatc cgcctacctc 7050
ggcctcccca agtgctggga ttagacgtga gccactgcgc ctggtcttct 7100
cactcattct tagacccagt gcaatctgac ttctctataa actactctga 7150
gatcaccagt aacctctaat tgtcaaacca tcaccctaca tggtatctg

TABLE 1C

The sequences shown in TABLE 1C, are sequence fragments taken from the protein C sequence shown in TABLE 1A above. Furthermore, SEQ ID NO.: 3 corresponds to the sequence underlined in TABLE 1A above. The nucleotide Y, at position 8 in SEQ ID NO.: 3 corresponds to the nucleotide found at position 4732 of SEQ ID NO.: 1. In all of the Sequences found in TABLE 1C below the polymorphism represented by a Y may substituted by an t or c. Furthermore, **bold** and underlined nucleotides represented by Y in SEQ ID NOs.: 4-12 in TABLE 1C, all correspond to the nucleotide found at position 4732 of SEQ ID NO.: 1. Due to the potential variability in protein C sequence, the sequence motifs below may be useful in identifying protein C sequences from a patient that are suitable for genotype determination. For Example, patient sequences that form alignments with the below motifs (SEQ ID NO.: 3-12) may indicate that the patient sequence is a protein C sequence and that the **bold** and underlined Y corresponds to the polymorphism at position 4732 of SEQ ID NO.: 1 and is therefore suitable for genotype determination. A similar strategy may be applied to the other polymorphism sites identified herein.

SEQ ID. NO.	SEQUENCE
SEQ ID. NO. 3	gcctttYcc cccatccctt
SEQ ID. NO. 4	aggatgcctt tYcccccatc
SEQ ID. NO. 5	Ycccccatcc cttcttgctc
SEQ ID. NO. 6	Ycccccatcc cttcttgctc acacccccaa
SEQ ID. NO. 7	cctcctctag gatgcctttY
SEQ ID. NO. 8	Tcaggcatac cctcctctag gatgcctttY
SEQ ID. NO. 9	gctcaggcat accctcctct aggatgcctt tY
SEQ ID. NO. 10	gctcaggcat accctcctct aggatgcctt tYcccccatc ccttcttgct cacaccccca acttgatctc tccctcctaa
SEQ ID. NO. 11	aggatgcctt tY
SEQ ID. NO. 12	gcctttYccc ccatcccttc

TABLE 1D

The sequences shown in TABLE 1D, are sequence fragments taken from the EPCR sequence shown in TABLE 1B above. Furthermore, SEQ ID NO.: 13 corresponds to the sequence underlined in TABLE 1B above. The nucleotide S, at position 8 in SEQ ID NO.: 13 corresponds to the nucleotide found at position 6196 of SEQ ID NO.: 2. In all of the sequences found in TABLE 1D below the polymorphism represented by an S may substituted by a "g" or "c". Furthermore, **bold** and underlined nucleotides represented by S in SEQ ID NOs.: 14-22 in TABLE 1D, all correspond to the nucleotide found at position 6196 of SEQ ID NO.: 2. Due to the potential variability in EPCR sequence, the sequence motifs below may be useful in identifying EPCR sequences from a patient that are suitable for genotype determination. For Example, patient sequences that form alignments with the below motifs (SEQ ID NO.: 13-22) may indicate that the patient sequence is an EPCR sequence and that the **bold** and underlined S corresponds to the polymorphism at position 6196 of SEQ ID NO.: 2 and is therefore suitable for genotype determination. A similar strategy may be applied to the other polymorphism sites identified herein.

SEQ ID. NO.	SEQUENCE
SEQ ID. NO. 13	cagcccc**S**tc agaaggggct
SEQ ID. NO. 14	tctccagccc c**S**tcagaagg
SEQ ID. NO. 15	**S**tcagaaggg gctggattga
SEQ ID. NO. 16	**S**tcagaaggg gctggattga tggaggctgg
SEQ ID. NO. 17	ttaattactc tccagcccc**S**
SEQ ID. NO. 18	gacggcgatg ttaattactc tccagcccc**S**
SEQ ID. NO. 19	gcgatgttaa ttactctcca gccccStcag aaggggctgg attgatggag
SEQ ID. NO. 20	tgtaggcatc ttcctgtgca caggtggacg gcgatgttaa ttactctcca gccccStcag aaggggctgg attgatggag gctggcaagg gaaagtttca
SEQ ID. NO. 21	tctccagccc c**S**
SEQ ID. NO. 22	agccccStca gaaggggctg

An "allele" is defined as any one or more alternative forms of a given gene. In a diploid cell or organism the members of an allelic pair (i.e. the two alleles of a given gene) occupy corresponding positions (loci) on a pair of homologous chromosomes and if these alleles are genetically identical the cell or organism is said to be "homozygous", but if genetically different the cell or organism is said to be "heterozygous" with respect to the particular gene.

A "gene" is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product and may include untranslated and untranscribed sequences in proximity to the coding regions. Such non-coding sequences may contain regulatory sequences needed for transcription and translation of the sequence or introns etc.

A "genotype" is defined as the genetic constitution of an organism, usually in respect to one gene or a few genes or a region of a gene relevant to a particular context (i.e. the genetic loci responsible for a particular phenotype).

A "phenotype" is defined as the observable characters of an organism.

A "single nucleotide polymorphism" (SNP) occurs at a polymorphic site occupied by a single nucleotide, which is the site of variation between allelic sequences. The site is usually preceded by and followed by highly conserved sequences of the allele (e.g., sequences that vary in less than 1/100 or 1/1000 members of the populations). A single nucleotide polymorphism usually arises due to substitution of one nucleotide for another at the polymorphic site. A "transition" is the replacement of one purine by another purine or

one pyrimidine by another pyrimidine. A "transversion" is the replacement of a purine by a pyrimidine or vice versa. Single nucleotide polymorphisms can also arise from a deletion (represented by "-" or "*del*") of a nucleotide or an insertion (represented by "+" or "*ins*") of a nucleotide relative to a reference allele. Furthermore, it would be appreciated by a person of skill in the art, that an insertion or deletion within a given sequence could alter the relative position and therefore the position number of another polymorphism within the sequence.

A "systemic inflammatory response syndrome" or (SIRS) is defined as including both septic (i.e. sepsis or septic shock) and non-septic systemic inflammatory response (i.e. post operative). "SIRS" is further defined according to ACCP (American College of Chest Physicians) guidelines as the presence of two or more of A) temperature > 38°C or < 36°C, B) heart rate > 90 beats per minute, C) respiratory rate > 20 breaths per minute, and D) white blood cell count > 12,000 per mm3 or < 4,000 mm3. In the following description, the presence of two, three, or four of the "SIRS" criteria were scored each day over the 28 day observation period.

"Sepsis" is defined as the presence of at least two "SIRS" criteria and known or suspected source of infection. Septic shock was defined as sepsis plus one new organ failure by Brussels criteria plus need for vasopressor medication.

Patient outcome or prognosis as used herein refers the ability of a patient to recover from an inflammatory condition. An inflammatory condition, may be selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumanitis, infection, pancreatitis, bacteremia, peritonitis, abdominal

abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglotittis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

Assessing patient outcome or prognosis may be accomplished by various methods. For Example, an "APACHE II" score is defined as Acute Physiology And Chronic Health Evaluation and herein was calculated on a daily basis from raw clinical and laboratory

variables. Vincent *et al.* (Vincent JL. Ferreira F. Moreno R. *Scoring systems for assessing organ dysfunction and survival.* Critical Care Clinics. 16:353-366, 2000) summarize APACHE score as follows "First developed in 1981 by Knaus *et al.*, the APACHE score has become the most commonly used survival prediction model in ICUs worldwide. The APACHE II score, a revised and simplified version of the original prototype, uses a point score based on initial values of 12 routine physiologic measures, age, and previous health status to provide a general measure of severity of disease. The values recorded are the worst values taken during the patient's first 24 hours in the ICU. The score is applied to one of 34 admission diagnoses to estimate a disease-specific probability of mortality (APACHE II predicted risk of death). The maximum possible APACHE II score is 71, and high scores have been well correlated with mortality. The APACHE II score has been widely used to stratify and compare various groups of critically ill patients, including patients with sepsis, by severity of illness on entry into clinical trials." Furthermore , the criteria or indication for administering activated protein C (XIGRIS™ -drotrecogin alfa (activated)) in the United States is an APACHE II score of ≥25. In Europe, the criteria or indication for administering activated protein C is an APACHE II score of ≥25 or 2 organ system failures.

"Activated protein C" is also known as Drotrecogin alfa (activated) and is sold as XIGRIS™ by Eli Lilly and Company. Drotrecogin alfa (activated) is a serine protease glycoprotein of approximately 55 kilodalton molecular weight and having the same amino acid sequence as human plasma-derived Activated Protein C. The protein consists of a heavy chain and a light chain linked by a disulfide bond. XIGRIS™, Drotecogin alfa (activated) is indicated for the reduction of mortality in adult patients with severe sepsis (sepsis associated with acute organ dysfunction) who have a high risk of death (e.g., as determined by an APACHE II score of greater > 25 or having 2 or more organ system failures).

XIGRIS™ is available in 5 mg and 20 mg single-use vials containing sterile, preservative-free, lyophilized drug. The vials contain 5.3 mg and 20.8 mg of drotrecogin alfa (activated), respectively. The 5 and 20 mg vials of XIGRIS™ also contain 40.3 and 158.1 mg of sodium chloride, 10.9 and 42.9 mg of sodium citrate, and 31.8 and 124.9 mg of sucrose, respectively. XIGRIS™ is recoomended for intravenous administration at an

infusion rate of 24 mcg/kg/hr for a total duration of infusion of 96 hours. Dose adjustment based on clinical or laboratory parameters is not recommended. If the infusion is interrupted, it is recommended that when restarted the infusion rate should be 24 mcg/kg/hr. Dose escalation or bolus doses of drotrecogin alfa are not recommended. XIGRIS™ may be reconstituted with Sterile Water for Injection and further diluted with sterile normal saline injection. These solutions must be handled so as to minimize agitation of the solution (Product information. XIGRIS™, Drotecogin alfa (activated), Eli Lilly and Company, November 2001).

Drotrecogin alfa (activated) is a recombinant form of human Activated Protein C, which may be produced using a human cell line expressing the complementary DNA for the inactive human Protein C zymogen, whereby the cells secrete protein into the fermentation medium. The protein may be enzymatically activated by cleavage with thrombin and subsequently purified. Methods, DNA compounds and vectors for producing recombinant activated human protein C are described in US patents 4,775,624; 4,992,373; 5,196,322; 5,270,040; 5,270,178; 5,550,036; 5,618,714 all of which are incorporated herein by reference.

Treatment of sepsis using activated protein C in combination with a bactericidal and endotoxin neutralizing agent is described in US patent 6,436,397;methods for processing protein C is described in US patent 6,162,629; protein C derivatives are described in US patents 5,453,373 and 6,630,138; glycosylation mutants are described in US patent 5,460,953; and Protein C formulations are described in US patents 6,630,137, 6,436,397, 6,395,270 and 6,159,468, all of which are incorporated herein by reference.

A "Brussels score" score is a method for evaluating organ dysfunction as compared to a baseline. If the Brussels score is 0 (ie. moderate, severe, or extreme), then organ failure was recorded as present on that particular day (see TABLE 2A below). In the following description, to correct for deaths during the observation period, days alive and free of organ failure (DAF) were calculated as previously described. For example, acute lung injury was calculated as follows. Acute lung injury is defined as present when a patient meets all of these four criteria. 1) Need for mechanical ventilation, 2) Bilateral pulmonary infiltrates on chest X-ray consistent with acute lung injury, 3) PaO_2/FiO_2 ratio is less than

300, 4) No clinical evidence of congestive heart failure or if a pulmonary artery catheter is in place for clinical purposes, a pulmonary capillary wedge pressure less than 18 mm Hg (1). The severity of acute lung injury is assessed by measuring days alive and free of acute lung injury over a 28 day observation period. Acute lung injury is recorded as present on each day that the person has moderate, severe or extreme dysfunction as defined in the Brussels score. Days alive and free of acute lung injury is calculated as the number of days after onset of acute lung injury that a patient is alive and free of acute lung injury over a defined observation period (28 days). Thus, a lower score for days alive and free of acute lung injury indicates more severe acute lung injury. The reason that days alive and free of acute lung injury is preferable to simply presence or absence of acute lung injury, is that acute lung injury has a high acute mortality and early death (within 28 days) precludes calculation of the presence or absence of acute lung injury in dead patients. The cardiovascular, renal, neurologic, hepatic and coagulation dysfunction were similarly defined as present on each day that the person had moderate, severe or extreme dysfunction as defined by the Brussels score. Days alive and free of steroids are days that a person is alive and is not being treated with exogenous corticosteroids (e.g. hydrocortisone, prednisone, methylprednisolone). Days alive and free of pressors are days that a person is alive and not being treated with intravenous vasopressors (e.g. dopamine, norepinephrine, epinephrine, phenylephrine). Days alive and free of an International Normalized Ratio (INR) > 1.5 are days that a person is alive and does not have an INR > 1.5.

TABLE 2A

Brussels Organ Dysfunction Scoring System

ORGANS	Free of Organ Dysfunction		Clinically Significant Organ Dysfunction		
	Normal	Mild	Moderate	Severe	Extreme
DAF ORGAN DYSFUNCTION SCORE	**1**		**0**		
Cardiovascular Systolic BP (mmHg)	>90	≤90 Responsive to fluid	≤90 Unresponsive to fluid	≤90 plus pH ≤7.3	≤90 plus pH ≤7.2
Pulmonary P_aO_2/F_{IO_2} (mmHg)	>400	400-301	300-201 Acute lung injury	200-101 ARDS	≤100 Severe ARDS

Renal Creatinine (mg/dL)	<1.5	1.5-1.9	2.0-3.4	3.5-4.9	≥5.0
Hepatic Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Hematologic Platelets ($x10^5/mm^3$)	>120	120-81	80-51	50-21	≤20
Neurologic (Glascow Score)	15	14-13	12-10	9-6	≤5
Round Table Conference on Clinical Trials for the Treatment of Sepsis Brussels, March 12-14, 1994.					

Analysis of variance (ANOVA) is a standard statistical approach to test for statistically significant differences between sets of measurements.

The Fisher exact test is a standard statistical approach to test for statistically significant differences between rates and proportions of characteristics measured in different groups.

2. General Methods

One aspect of the invention may involve the identification of patients or the selection of patients that are either at risk of developing and inflammatory condition or the identification of patients who already have an inflammatory condition. For example, patients who have undergone major surgery or scheduled for or contemplating major surgery may be considered as being at risk of developing an inflammatory condition. Furthermore, patients may be determined as having an inflammatory condition using diagnostic methods and clinical evaluations known in the medical arts. An inflammatory condition, may be selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumanitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due

to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglotittis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

Once a patient is identified as being at risk for developing or having an inflammatory condition, then genetic sequence information may be obtained from the patient. Or alternatively genetic sequence information may already have been obtained from the patient. For example, a patient may have already provided a biological sample for other purposes or may have even had their genetic sequence determined in whole or in part and

stored for future use. Genetic sequence information may be obtained in numerous different ways and may involve the collection of a biological sample that contains genetic material. Particularly, genetic material, containing the sequence or sequences of interest. Many methods are known in the art for collecting bodily samples and extracting genetic material from those samples. Genetic material can be extracted from blood, tissue and hair and other samples. There are many known methods for the separate isolation of DNA and RNA from biological material. Typically, DNA may be isolated from a biological sample when first the sample is lysed and then the DNA is isolated from the lysate according to any one of a variety of multi-step protocols, which can take varying lengths of time. DNA isolation methods may involve the use of phenol (Sambrook, J. *et al.*, "Molecular Cloning", Vol. 2, pp. 9.14-9.23, Cold Spring Harbor Laboratory Press (1989) and Ausubel, Frederick M. *et al.*, "Current Protocols in Molecular Biology", Vol. 1, pp. 2.2.1-2.4.5, John Wiley & Sons, Inc. (1994)). Typically, a biological sample is lysed in a detergent solution and the protein component of the lysate is digested with proteinase for 12-18 hours. Next, the lysate is extracted with phenol to remove most of the cellular components, and the remaining aqueous phase is processed further to isolate DNA. In another method, described in Van Ness *et al.* (U.S. Pat. # 5,130,423), non-corrosive phenol derivatives are used for the isolation of nucleic acids. The resulting preparation is a mix of RNA and DNA.

Other methods for DNA isolation utilize non-corrosive chaotropic agents. These methods, which are based on the use of guanidine salts, urea and sodium iodide, involve lysis of a biological sample in a chaotropic aqueous solution and subsequent precipitation of the crude DNA fraction with a lower alcohol. The final purification of the precipitated, crude DNA fraction can be achieved by any one of several methods, including column

chromatography (Analects, (1994) Vol 22, No. 4, Pharmacia Biotech), or exposure of the crude DNA to a polyanion-containing protein as described in Koller (U.S. Pat. # 5,128,247).

Yet another method of DNA isolation, which is described by Botwell, D. D. L. (Anal. Biochem. (1987) 162:463-465) involves lysing cells in 6M guanidine hydrochloride, precipitating DNA from the lysate at acid pH by adding 2.5 volumes of ethanol, and washing the DNA with ethanol.

Numerous other methods are known in the art to isolate both RNA and DNA, such as the one described by Chomczynski (U.S. Pat. # 5,945,515), whereby genetic material can be extracted efficiently in as little as twenty minutes. Evans and Hugh (U.S. Pat. # 5,989,431) describe methods for isolating DNA using a hollow membrane filter.

Once a patient's genetic sequence information has been obtained from the patient it may then be further analyzed to detect or determine the identity or genotype of one or more polymorphisms in the protein C gene. Provided that the genetic material obtained, contains the sequence of interest. Particularly, a person may be interested in determining the protein C genotype of a patient of interest, where the genotype includes a nucleotide corresponding to position 4732 or SEQ ID NO.: 1 or position 8 of SEQ ID NO.: 3. The sequence of interest may also include other protein C gene polymorphisms or may also contain some of the sequence surrounding the polymorphism of interest. Detection or determination of a nucleotide identity or the genotype of the single nucleotide polymorphism(s) or other polymorphism, may be accomplished by any one of a number methods or assays known in the art, including but not limited to the following:

Restriction Fragment Length Polymorphism (RFLP) strategy – An RFLP gel-based analysis can be used to distinguish between alleles at polymorphic sites within a gene. Briefly, a short segment of DNA (typically several hundred base pairs) is amplified by PCR. Where possible, a specific restriction endonuclease is chosen that cuts the short DNA segment when one variant allele is present but does not cut the short DNA segment when the other allele variant is present. After incubation of the PCR amplified DNA with this restriction endonuclease, the reaction products are then separated using gel electrophoresis. Thus, when the gel is examined the appearance of two lower molecular weight bands (lower molecular weight molecules travel farther down the gel during electrophoresis) indicates that the initial DNA sample had the allele which could be cut by the chosen restriction endonuclease. In contrast, if only one higher molecular weight band is observed (at the molecular weight of the PCR product) then the initial DNA sample had the allele variant that could not be cut by the chosen restriction endonuclease. Finally, if both the higher molecular weight band and the two lower molecular weight bands are visible then the initial DNA sample contained both alleles, and therefore the patient was heterozygous for this single nucleotide polymorphism;

Sequencing – For example the Maxam-Gilbert technique for sequencing (Maxam AM. and Gilbert W. Proc. Natl. Acad. Sci. USA (1977) 74(4):560-564) involves the specific chemical cleavage of terminally labelled DNA. In this technique four samples of the same labeled DNA are each subjected to a different chemical reaction to effect preferential cleavage of the DNA molecule at one or two nucleotides of a specific base identity. The conditions are adjusted to obtain only partial cleavage, DNA fragments are thus generated in each sample whose lengths

are dependent upon the position within the DNA base sequence of the nucleotide(s) which are subject to such cleavage. After partial cleavage is performed, each sample contains DNA fragments of different lengths, each of which ends with the same one or two of the four nucleotides. In particular, in one sample each fragment ends with a C, in another sample each fragment ends with a C or a T, in a third sample each ends with a G, and in a fourth sample each ends with an A or a G. When the products of these four reactions are resolved by size, by electrophoresis on a polyacrylamide gel, the DNA sequence can be read from the pattern of radioactive bands. This technique permits the sequencing of at least 100 bases from the point of labeling. Another method is the dideoxy method of sequencing was published by Sanger *et al.* (Sanger *et al.* Proc. Natl. Acad. Sci. USA (1977) 74(12):5463-5467). The Sanger method relies on enzymatic activity of a DNA polymerase to synthesize sequence-dependent fragments of various lengths. The lengths of the fragments are determined by the random incorporation of dideoxynucleotide base-specific terminators. These fragments can then be separated in a gel as in the Maxam-Gilbert procedure, visualized, and the sequence determined. Numerous improvements have been made to refine the above methods and to automate the sequencing procedures. Similary, RNA sequencing methods are also known. For example, reverse transcriptase with dideoxy-nucleotides have been used to sequence encephalomyocarditis virus RNA (Zimmern D. and Kaesberg P. Proc. Natl. Acad. Sci. USA (1978) 75(9):4257-4261). Mills DR. and Kramer FR. (Proc. Natl. Acad. Sci. USA (1979) 76(5):2232-2235) describe the use of Q.beta. replicase and the nucleotide analog inosine for sequencing RNA in a chain-termination mechanism. Direct chemical methods for sequencing RNA are also known (Peattie DA. Proc. Natl. Acad. Sci. USA (1979)

76(4):1760-1764). Other methods include those of Donis-Keller *et al.* (1977, Nucl. Acids Res. 4:2527-2538), Simoncsits A. *et al.* (Nature (1977) 269(5631):833-836), Axelrod VD. *et al.* (Nucl. Acids Res.(1978) 5(10):3549-3563), and Kramer FR. and Mills DR. (Proc. Natl. Acad. Sci. USA (1978) 75(11):5334-5338, which are incorporated herein by reference). Nucleic acid sequences can also be read by stimulating the natural fluoresce of a cleaved nucleotide with a laser while the single nucleotide is contained in a fluorescence enhancing matrix (U.S. Pat. # 5,674,743);

Hybridization methods for the identification of SNPs using hydridization techniques are described in the U.S. Pat. # 6,270,961 & 6,025,136;

A template-directed dye-terminator incorporation with fluorscent polarization-detection (TDI-FP) method is described by FREEMAN BD. et al. (J Mol Diagnostics (2002) 4(4):209-215) is described for large scale screening;

Oligonucleotide ligation assay (OLA) - is based on ligation of probe and detector oligonucleotides annealed to a polymerase chain reaction amplicon strand with detection by an enzyme immunoassay (VILLAHERMOSA ML. J Hum Virol (2001) 4(5):238-48; ROMPPANEN EL. Scand J Clin Lab Invest (2001) 61(2):123-9; IANNONE MA. *et al.* Cytometry (2000) 39(2):131-40);

Ligation-Rolling Circle Amplification (L-RCA) has also been successfully used for genotyping single nucleotide polymorphisms as described in QI X. *et al.* Nucleic Acids Res (2001) 29(22):E116;

5′ nuclease assay has also been successfully used for genotyping single nucleotide polymorphisms (AYDIN A. *et al.* Biotechniques (2001) (4):920-2, 924, 926-8.);

Polymerase proofreading methods are used to determine SNPs identities, as described in WO 0181631;

Detection of single base pair DNA mutations by enzyme-amplified electronic transduction is described in PATOLSKY F et al. Nat Biotech. (2001) 19(3):253-257;

Gene chip technologies are also known for single nucleotide polymorphism discrimination whereby numerous polymorphisms may be tested for simultaneously on a single array (EP 1120646 and Gilles PN. et al. Nat. Biotechnology (1999) 17(4):365-70);

Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectroscopy is also useful in the genotyping single nucleotide polymorphisms through the analysis of microsequencing products (Haff LA. and Smirnov IP. Nucleic Acids Res. (1997) 25(18):3749-50; Haff LA. and Smirnov IP. Genome Res. (1997) 7:378-388; Sun X. et al. Nucleic Acids Res. (2000) 28 e68; Braun A. et al. Clin. Chem. (1997) 43:1151-1158; Little DP. et al. Eur. J. Clin. Chem. Clin. Biochem. (1997) 35:545-548; Fei Z. et al. Nucleic Acids Res. (2000) 26:2827-2828; and Blondal T. et al. Nucleic Acids Res. (2003) 31(24):e155); or

Allele specific PCR methods have also been successfully used for genotyping single nucleotide polymorphisms (Hawkins JR. *et al.* Hum Mutat (2002) 19(5):543-553).

Alternatively, if a patient's sequence data is already known, then obtaining may involve retrieval of the patients nucleic acid sequence data from a database, followed by determining or detecting the identity of a nucleic acid or genotype at a polymorphism site by reading the patient's nucleic acid sequence at the polymorphic site.

Once the identity of a polymorphism(s) is determined or detected an indication may be obtained as to patient outcome or prognosis or ability of a patient recover from an inflammatory condition based on the genotype (the nucleotide at the position) of the polymorphism of interest. In the present invention, polymorphisms in protein C sequence and/or polymorphisms in endothelial cell protein C receptor (EPCR) sequence, are used to obtain a prognosis or to determine patient outcome. Methods for obtaining patient outcome or prognosis or for patient screening may be useful to determine the ability of a patient to recover from an inflammatory condition. Alternatively, single polymorphism sites or combined polymorphism sites may be used as an indication of a patient's ability to recover from an inflammatory condition, if they are linked to a polymorphism determined to be indicative of a patient's ability to recover from an inflammatory condition. The method may further comprise comparing the genotype determined for a polymorphism with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition. Accordingly, a decision regarding the patient's ability to recover may be from an inflammatory condition may be made based on the geneotype determined for the polymorphism site.

Once patient outcome or a prognosis is determined, such information may be of interest to physicians and surgeons to assist in deciding between potential treatment options, to help

determine the degree to which patients are monitored and the frequency with which such monitoring occurs. Ultimately, treatment decisions may be made in response to factors, both specific to the patient and based on the experience of the physician or surgeon responsible for a patient's care. Treatment options that a physician or surgeon may consider in treating a patient with an inflammatory condition may include, but are not limited to the following:

(a) use of anti-inflammatory therapy;

(b) use of steroids;

(c) use of activated Protein C (drotrocogin alpha or Xigris™ from Lilly);

(d) use of modulators of the coagulation cascade (such as various versions of heparin) use of antibody to tissue factor;

(e) use of anti-thrombin or anti-thrombin III;

(f) streptokinase;

(g) use of antiplatelet agents such as clopidegrel; and

(h) Surfactant.

Alternative treatments currently in development and potentially useful in the treatment of an inflammatory condition may include, but are not limited to the following: antibodies to tumor necrosis factor (TNF) or even antibody to endotoxin (i.e. lipopolysaccharide, LPS); tumor necrosis factor receptor (TNF); tissue factor pathway inhibitors (tifacogin™ alpha from Chiron); platelet activating factor hydrolase (PAFase™ from ICOS); antibodies to IL-6; antibodies, antagonists or inhibitors to high mobility group box 1 (HMGB-1 or HMG-1 tissue plasminogen activator; bradykinin antagonists; antibody to CD-14; interleukin-10; Recombinant soluble tumor necrosis factor receptor-immunoglobulin G1(Roche); Procysteine; Elastase Inhibitor; and human recombinant interleukin 1 receptor antagonist (IL-1 RA).

Methods of treatment of an inflammatory condition in a patient having one or more of the risk geneotypes in protein C and/or EPCR associated with improved response to a therapeutic agent are described herein. An improved response may include an improvement subsequent to administration of said therapeutic agent, whereby the patient has an increased likelihood of survival, reduced likelihood of organ damage or organ dysfunction (Brussels score), an improved APACHE II score, days alive and free of pressors, inotropes, and reduced systemic dysfunction (cardiovascular, respiratory, ventilation, CNS, coagulation [INR> 1.5], renal and/or hepatic).

As described above genetic sequence information or genotype information may be obtained from a patient wherein the sequence information contains one or more single nucleotide polymorphism sites in protein C sequence and/or EPCR sequence. Also, as previously described the sequence identity of one or more single nucleotide polymorphisms in the protein C sequence and EPCR sequence of one or more patients may then be detected or determined. Furthermore, patient outcome or prognosis may be assessed as described above, for example the APACHE II scoring system or the Brussels score may be used to assess patient outcome or prognosis by comparing patient scores before and after treatment. Once patient outcome or prognosis has been assessed, patient outcome or prognosis may be correlated with the sequence identity of one or more single nucleotide polymorphism(s). The correlation of patient outcome or prognosis may further include statistical analysis of patient outcome scores and polymorphism(s) for a number of patients.

Clinical Phenotype

The primary outcome variable was survival to hospital discharge. Secondary outcome variables were days alive and free of cardiovascular, respiratory, renal, hepatic, hematologic, and neurologic organ system failure as well as days alive and free of SIRS (Systemic Inflammatory Response Syndrome), occurrence of sepsis, and occurrence of septic shock. SIRS was considered present when patients met at least two of four SIRS criteria. The SIRS criteria were 1) fever (>38 °C) or hypothermia (<35.5 °C), 2) tachycardia (>100 beats/min in the absence of beta blockers, 3) tachypnea (>20 breaths/min) or need for mechanical ventilation, and 4) leukocytosis (total leukocyte count > 11,000/μL) (Anonymous. *Critical Care Medicine* (1992) 20(6):864-74). Patients were included in this cohort on the calendar day on which the SIRS criteria were met.

A patients' baseline demographics that were recorded included age, gender, whether medical or surgical diagnosis for admission (according to APACHE III diagnostic codes (KNAUS WA et al. *Chest* (1991) 100(6):1619-36)), and admission APACHE II score. The following additional data were recorded for each 24 hour period (8 am to 8 am) for 28 days to evaluate organ dysfunction, SIRS, sepsis, and septic shock.

Clinically significant organ dysfunction for each organ system was defined as present during a 24 hour period if there was evidence of at least moderate organ dysfunction using the Brussels criteria (TABLE 2A) (RUSSELL JA et al. *Critical Care Medicine* (2000) 28(10):3405-11). Because data were not always available during each 24 hour period for each organ dysfunction variable, we used the "carry forward" assumption as defined previously (Anonymous. *New England Journal of Medicine* (2000) 342(18):1301-8). Briefly, for any 24 hour period in which there was no measurement of a variable, we carried forward the "present" or "absent" criteria from the previous 24 hour period. If any variable was never measured, it was assumed to be normal.

To further evaluate cardiovascular, respiratory, and renal function we also recorded, during each 24 hour period, vasopressor support, mechanical ventilation, and renal support, respectively. Vasopressor use was defined as dopamine > 5 μg/kg/min or any dose of norepinephrine, epinephrine, vasopressin, or phenylephrine. Mechanical

ventilation was defined as need for intubation and positive airway pressure (i.e. T- piece and mask ventilation were not considered ventilation). Renal support was defined as hemodialysis, peritoneal dialysis, or any continuous renal support mode (e.g. continuous veno-venous hemodialysis).

To assess duration of organ dysfunction and to correct organ dysfunction scoring for deaths in the 28-day observation period, calculations were made of days alive and free of organ dysfunction (DAF) as previously reported (BERNARD GR et al. *New England Journal of Medicine* (1997) 336(13):912-8). Briefly, during each 24-hour period for each variable, DAF was scored as 1 if the patient was alive and free of organ dysfunction (normal or mild organ dysfunction, Table 1). DAF was scored as 0 if the patient had organ dysfunction (moderate, severe, or extreme) or was not alive during that 24-hour period. Each of the 28 days after ICU admission was scored in each patient in this fashion. Thus, the lowest score possible for each variable was zero and the highest score possible was 28. A low score is indicative of more organ dysfunction as there would be fewer days alive and free of organ dysfunction.

Similarly, days alive and free of SIRS (DAF SIRS) were calculated. Each of the four SIRS criteria were recorded as present or absent during each 24 hour period. Presence of SIRS during each 24 hour period was defined by having at least 2 of the 4 SIRS criteria. Sepsis was defined as present during a 24 hour period by having at least two of four SIRS criteria and having a known or suspected infection during the 24 hour period (Anonymous. *Critical Care Medicine* (1992) 20(6):864-74). Cultures that were judged to be positive due to contamination or colonization were excluded. Septic shock was defined as presence of sepsis plus presence of hypotension (systolic blood pressure < 90 mmHg or need for vasopressor agents) during the same 24 hour period.

Haplotypes and Selection of htSNPs

Using unphased Caucasian genotypic data (from **pga.mbt.washington.edu** (RIEDER MJ et al. SeattleSNPs. NHLBI Program for Genomic Applications, UW-FHCRC, Seattle, WA (2001)) haplotypes were inferred using PHASE (STEPHENS M. et al. *Am J Hum Genet* (2001) 68:978-89) software (Figures 1 and 2). MEGA 2 (KUMAR S. et al. (2001)

17:1244-5) was then used to infer a phylogenetic tree to identify major haplotype clades for EPCR (Figures 3). Haplotypes were sorted according to the phylogenetic tree and haplotype structure was inspected to choose haplotype tag SNPs (htSNPs) (JOHNSON GC. et al. *Nat Genet* (2001) 29:233-7; and GABRIEL SB. et al. *Science* (2002) 296:2225-9). htSNPs that identified major haplotype clades of EPCR in Caucasians were chosen. These SNPs were then genotyped in our patient cohort to define haplotypes and haplotype clades.

Blood Collection/Processing Genotyping

The buffy coat was extracted from whole blood and samples transferred into 1.5 ml cryotubes and stored at -80°C. DNA was extracted from the buffy coat of peripheral blood samples using a QIAamp DNA Blood Maxi Kit (Qiagen™). The genotypic analysis was performed in a blinded fashion, without clinical information. Polymorphisms were genotyped using either a Masscode tagging (Qiagen Genomics, Inc - KOKORIS M et al *Molecular Diagnosis* (2000) 5(4):329-40; BRAY MS. et al. *Hum Mutat* (2001) 17:296-304.).

3. EXAMPLES

EXAMPLE 1: EPCR Haplotype Analysis

Inclusion Criteria

498 consecutive critically ill patients admitted to St. Paul's Hospital Intensive Care Unit (ICU) met the inclusion criteria of having at least two out of four SIRS criteria and were included into our study.

Data Collection

Data was recorded for 28 days or until hospital discharge. Raw clinical and laboratory variables were recorded using the worst or most abnormal variable for each 24 hour period with the exception of Glasgow Coma Score, where the best possible score for each 24 hour period was recorded. Missing data on the date of admission was assigned a normal value and missing data after the day one was substituted by carrying forward the previous day's value. Demographic and microbiologic data were recorded. When data collection for each patient was complete, all patient identifiers were removed from all records and the patient file was assigned a unique random number that was cross referenced with the blood samples. The completed raw data file was converted to calculated descriptive and severity of illness scores using standard definitions (i.e. APACHE II and Days alive and free of organ dysfunction calculated using the Brussels criteria).

Statistical Analysis

We used a cohort study design. Rates of dichotomous outcomes (28-day mortality, sepsis and shock at onset of SIRS) were compared between haplotype clades using a chi-squared test, assuming a dominant model of inheritance. Differences in continuous outcome variables between haplotype clades were tested using ANOVA. 28-day mortality was further compared between haplotype clades while adjusting for other confounders (age, sex, and medical vs. surgical diagnosis) using a Cox regression model, together with Kaplan-Meier analysis. Haplotype clade relative risk was calculated. This analysis was performed in the entire cohort, and subsequently in sub-groups of patients who had sepsis at onset of SIRS, and patients who had septic shock at onset of SIRS. Genotype distributions were tested for Hardy-Weinberg equilibrium (GUO SW. and THOMPSON EA. (1992) 48:361-72). We report the mean and 95% confidence intervals. Statistical significance was set at $p < 0.05$. The data was analyzed using SPSS 11.5 for Windows™ and SigmaStat 3.0 software (SPSS Inc, Chicago, IL, 2003).

Seven haplotypes of the EPCR gene were infered using PHASE software as described above and phylogenetic analysis was used to sort these haplotypes into 3 clades (Figure 3). The htSNPs A6118G (rs867186) and G6196C (rs9574) to uniquely identify each haplotype clade (Figure 2). 222 Caucasian patients admitted to our ICU with SIRS and successfully genotyped for the A6118G and G6196C polymorphisms were included in this study. The genotype frequencies of A6118G and G6196C are shown in Table 3A. These alleles were in Hardy Weinberg equilibrium in our population. Haplotype clade 1, defined by 6118A/6196C, occurred with a frequency of 37%. Haplotype 2, defined by 6118A/6196G, occurred in 39% of our cohort, while haplotype 3, defined by 6118G/6196G, occurred in 24% of our cohort.

TABLE 3A
Genotype frequencies of EPCR haplotype tag SNPs A6118G and C6196G

	Genotype Frequencies			Allele Frequencies		p*
	AA	AG	GG	A	G	
A6118G	81%	19%	0%	90.5%	9.5%	0.99
G6196C	CC	CG	GG	C	G	
	23%	41%	36%	44%	56%	0.98

* Chi-Squared test for Hardy-Weinberg equilibrium

TABLE 3B
Genotype frequencies of EPCR haplotype tag SNP T4054C

	Genotype Frequencies			Allele Frequencies		p*
	TT	CT	CC	T	C	
T4054C	30%	50%	20%	55%	45%	0.99

*Chi-Squared test for Hardy-Weinberg equilibrium

Table 4 shows that there were no significant differences in baseline characteristics of associated with haplotype clades 1, 2, or 3. Patients were of similar age had similar APACHE II scores. There was a trend to more males in haplotype 3 (Table 4). There was no difference in the frequency of sepsis or septic shock at the time of onset of SIRS (Table 4).

TABLE 4

Baseline characteristics and mortality of 222 critically ill patients who had SIRS

Haplotype Clade	Mean Age	Gender (% Male)	Diagnosis for admission (% Surgical)	Mean APACHE II	Sepsis on Admission	Septic Shock On Admission	28-day Mortality
1	61	63%	30%	20	54%	43%	31%
2	59	65%	31%	19	61%	50%	37%
3	63	79%	33%	20	60%	52%	33%
p	NS	0.06	NS	NS	NS	NS	NS

The EPCR haplotype clades 2 and 3 were associated with fewer days alive and free of acute lung injury /ARDS injury than haplotype clade 1 (Figure 4) in our entire cohort of patients with SIRS. There was also a trend ($p < 0.07$) to more acute renal dysfunction (expressed as fewer days alive and free of acute renal dysfunction) in haplotype clades 2 and 3. These associations were not seen in sub-groups of patients with sepsis at onset of SIRS, or those patients with septic shock at onset of SIRS.

There was no difference between between haplotype clades 1, 2 or 3 in 28 day mortality (Table 4). There were no associations of EPCR haplotypes with cardiovascular, neurologic, hepatic or coagulation dysfunction (Table 5). There was also no association of haplotype or genotype with days alive and free of ventilatory, vasopressor or renal support (Table 6).

TABLE 5

Days alive and free of (DAF) SIRS and Key Organ Dysfunction in 222 critically ill patients who had SIRS

Haplotype	DAF SIRS 4/4	DAF SIRS 3/4	DAF ALI	DAF CNS	DAF CVS	DAF COAG	DAF RENAL	DAF HEPATIC
1	22.	22	20	21	21	24	19	20
2	20	20	17	19	19	24	17	19
3	21	21	18	20	20	25	18	20
p	NS	NS	0.006	NS	NS	NS	0.07	NS

TABLE 6

Days alive and free of (DAF) Life Support in 222 critically ill patients who had SIRS

Haplotype	DAF Vasopressors	DAF Renal Support	DAF Ventilatory Support
1	19	19	15
2	18	18	14
3	19	19	15

p	NS	NS	NS

When examined individually, it was found that neither htSNP was associated with a difference in baseline characteristics (age, sex, medical vs. surgical diagnosis, APACHE II score), 28-day mortality, or days alive and free of organ dysfunction, with the exception of acute lung injury. The EPCR 6196 G/G genotype was associated with significantly fewer days alive and free of acute lung injury/ARDS than the 6196G/C and C/C genotypes combined (16 days vs. 20 days, $p<0.006$), again indicating more acute lung injury/ARDS. The 6196 G allele is contained within both haplotype clades 2 and 3.

EXAMPLE 2: Patient Outcome or Prognosis for 4732 Protein C Polymorphisms

Table 7 shows the genotype frequencies of T4732C. These alleles were in Hardy Weinberg equilibrium in our population.

TABLE 7

Genotype frequencies of ProC haplotype tag SNP T4732C

	Genotype Frequencies			Allele Frequencies		p*
	TT	CT	CC	T	C	
T4732C	57%	37%	6%	76%	24%	0.99

*Chi-Squared test for Hardy-Weinberg equilibrium

It was found that SNP haplotypes of protein C 4732 are associated with altered survival and organ dysfunction in critically ill adults who have systemic inflammatory response syndrome (SIRS).

We studied an inception cohort of 489 Caucasian patients in ICU who met at least 2/4 criteria for SIRS and defined subgroups of patients who had sepsis or septic shock. Baseline variables were age, gender, APACHE II and medical vs. surgical reason for ICU admission. We determined 28-day survival (Kaplan Meier) and scored severity of organ dysfunction (by Brussels score) by calculating days alive and free (DAF) of organ dysfunction (respiratory, acute lung injury, cardiovascular, vasopressors, renal, coagulation, International Normalized Ratio for Partial Thromboplastin Time (INR), hepatic, and neurological (CNS) as well as systemic inflammatory response syndrome (SIRS with all 4 of 4 criteria (SIRS 4 of 4))) over 28 days. PHASE and MEGA 2 were used to determine the haplotypes of protein C in Caucasians. We then genotyped haplotype tag SNP's that tagged each of the major haplotype clades of each patient.

Patients were well matched by genotype and haplotype at baseline. We found that there were 3 major haplotype clades of protein C (xx, yy, zz %).

A novel clade was tagged by protein C T 4732 C and was associated with decreased 28-day survival (54 %, 60 % vs. 68 %, 4732 CC, CT, and TT respectively, $p < 0.05$ by Fisher's Exact Test) and with increased severity (measured as fewer DAF) of vasopressor use, renal, coagulation (platelets), INR, and hepatic dysfunction (all preceding have $p<0.05$) as well as more severe renal dysfunction (Spearman's rho) (See Table 8 below).

Table 8. Days alive and free (DAF) of vasopressors, coagulation (platelets), INR, renal, hepatic SIRS 4 of 4 and neurological (CNS) dysfunction in critically ill patients who had Systemic Inflammatory Response Syndrome (SIRS)

Genotype of Protein C 4732	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal
PC 4732 TT	18.8±11.1	19.8±11.1	19.1±11.3	17.8±11.6
PC 4732 CT	16.9±11.4	18.5±11.2	18±11.4	15.7±12.2
PC 4732 CC	15.6±11.2	16.9±10.6	16.8±10.9	15.8±10.5
P value	<0.05	<0.06	<0.05	<0.10

	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation
Genotype of Protein C 4732	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	
PC 4732 TT	20.1±11	19.5±10.4	19.3±11	
PC 4732 CT	18.8±11.2	18±10.7	17.9±11.4	
PC 4732 CC	15.5±12	16.2±10.3	16.1±11.7	
p	<0.06	<0.05	<0.11	
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	

The association of protein C 4732 C with decreased 28 day survival (57 % vs. 68%, protein C 4732 CC vs. protein C 4732 CT,TT, $p < 0.05$ by Kaplan Meier) and increased organ dysfunction (use of vasopressors, coagulation (platelets), INR, renal, hepatic SIRS 4 of 4, neurological (CNS) dysfunction and use of inotropic agents (inotropes) was especially pronounced in patients (n= 395 Caucasians) who had sepsis (See Table 9 below).

Table 9. Days alive and free (DAF) of vasopressors, coagulation (platelets), INR, renal, hepatic dysfunction, SIRS 4 of 4 crieria, neurological (CNS) dysfunction and use of inotropic agents (Inotropes) in critically ill patients who had Sepsis

Genotype of Protein C 4732	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal
PC 4732 TT	18.6±10.9	20.2±10.7	19.3±11.1	18±11.3
PC 4732 CT	16±11.3	17.8±11.3	17.3±11.4	14.9±12
PC 4732 CC	15.9±10.6	17.2±10.1	17±10.4	15.8±9.9
P value	<0.01	<0.01	<0.012	<0.02
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	DAF Inotropes
PC 4732 TT	20.4±10.7	19.4±10.1	19.4±10.5	20.7±10.3
PC 4732 CT	18±11.1	17.2±10.6	17±11.4	18.6±11.3
PC 4732 CC	15.4±11.9	16.4±9.6	16.3±11.3	19.5±10.3
P value	<0.008	<0.01	<0.06	<0.05
	Mean±Standa	Mean±Standard	Mean±Standa	

	rd Deviation	Deviation	rd Deviation	

A novel clade of protein C tagged by protein C 4732 C is a useful predictor decreased survival and increased multiple organ dysfunctions in SIRS and in sepsis.

EXAMPLE 3: Combination of EPCR and Protein C Polymorphisms

An interaction of novel haplotypes of protein c (protein C 4732 c) and endothelial protein C receptor (EPCR 4054 t) is associated with decreased survival and increased organ dysfunction in sirs, sepsis and septic shock

Patients who had no copies of the risk EPCR allele (4054T) and no copies of the adverse protein C allele (4732C) had the best 28 day survival and the least severity of organ dysfunction (protective-protective). Furthermore, patients who had at least one copy of the adverse EPCR allele (4054T) and at least one copy of the adverse protein C allele (4732C) had the lowest survival and the greatest organ dysfunction (risk-risk). Finally, patients who had either no copies of the adverse EPCR allele (4054T) and at least one copy of the adverse protein C allele (4732C) or who had at least one copy of the adverse EPCR allele (4054T) and no copies of the adverse protein C allele (4732C) had intermediate survival and organ dysfunction. These findings are interesting and suggest that the interaction of SNP haplotypes of protein C and EPCR are important predictors of the outcomes of critically ill patients who have SIRS.

Our results cannot be explained by differences in the baseline characteristics of the patients classified into our groups 1, 2 and 3 as there were no differences in important predictors of outcome including age, APACHE II score, proportion of patients who had sepsis at onset of the study and proportion of patients who had septic shock at the onset of the study.

Previously it was not known whether interactions of risk alleles of protein C and risk alleles of EPCR were associated with altered outcomes in systemic inflammatory response syndrome (SIRS) or sepsis. We show that interactions of alleles of protein C and EPCR

that are associated with increased risk of poor outcome ("risk alleles") is associated with increased risk of death and organ dysfunction in systemic inflammatory response syndrome (SIRS), sepsis and septic shock.

Our study was based on an inception cohort of 487 critically ill Caucasian patients who met at least 2/4 SIRS criteria. We defined subgroups who had sepsis (n= 393) and who had septic shock (n = 260). Outcomes were 28-day survival and severity of organ dysfunction by calculating days alive and free (DAF) of organ dysfunction (Brussels score: respiratory, cardiovascular, renal, coagulation, International Normalized Ratio for Partial Thromboplastin Time (INR) < 1.5, hepatic, and neurological dysfunction and use of vasopressors, inotropic agents, and renal support by continuous renal replacement therapy or dialysis (renal support)). Haplotypes and clades of protein C and EPCR were determined by PHASE and MEGA 2 in Caucasians. We selected haplotype tag SNP's that tagged each haplotype clade. We previously found novel haplotypes with risk alleles of protein C (tagged by 4732 C) and EPCR (4054 T) associated with increased risk of death and organ dysfunction. Therefore, we classified patients into 3 groups as having copies of protein C and EPCR risk alleles defined as follows:

Risk – Risk Group 1: defined patients who had at least 1 copy of the risk allele of protein C 4732 C and at least 1 copy of the EPCR 4054 T.

Risk – Protective Group 2: defined patients who had no risk alleles of protein C 4732 C and at last 1 copy of EPCR 4054 T OR at least 1 copy of the protein C 4732 C and no copies of the EPCR 4054 T.

Protective – Protective Group 3: defined patients who had no copies of the protein C 4732 C and no copies of the EPCR 4054 T (wild type).

EPCR	SNP 4054	Designation
	4054T	Risk
	4054C	Protective

ProC	SNP 4732	Designation
	4732C	Risk
	4732T	Protective

We then tested for associations of these 3 risk groups (Risk-Risk; Risk-Protective; Protective-Protective) with 28 day survival and with organ dysfunction as scored by days alive and free of organ dysfunction.

Patients with SIRS in the Protective-Protective Group had 28 day survival of 73.7 %, patients in the Risk-Protective Group had 28 day survival of 67 %, and patients in the Risk-Risk Group had 28 day survival of 58.4 % ($p < 0.02$ by Chi- square; $p < 0.03$ by Kaplan-Meier survival analysis over 28 days).

The organ dysfunction of patients who had SIRS according to group is shown in Table 10. There was a steady increase in organ dysfunction (scored as lower days alive and free of organ dysfunction and support) from Protective-Protective, through Risk-Protective to Risk-Risk groups.

Table 10. Days alive and free (DAF) of use of vasopressors, coagulation (platelets) dysfunction, INR, renal, cardiovascular dysfunction, hepatic dysfunction, SIRS 4 of 4, neurological (CNS) dysfunction and use of inotropic agents (inotropes) in 487 critically ill patients who had Systemic Inflammatory Response Syndrome (SIRS) according to group

PC 4732 C/EPCR 4054T Risk Group	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal	DAF CVS
Risk-Risk	16.1±11.3	18±11.2	17.6±11.5	15.2±12	14.5±11.2
Risk-Protective	18.5±11.3	19.5±11.3	19±11.4	17.6±11.6	16.1±11.2
Protective-Protective	20.4±10.4	21.5±10	20±10.5	19.5±11.2	18.8±10.6
P value	<0.003	<0.06	<0.05	<0.10	<0.018
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

PC 4732 C/EPCR 4054T Risk Group	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	DAF Inotropes
Risk-Risk	18.1±11.3	17.3±10.6	17.2±11.5	18.8±11.3

Risk-Protective	19.8±11.1	19.2±10.5	19±11	20.2±10.7
Protective-Protective	20.8±11.1	21±10	21±10.4	22.1±9.8
P value	<0.06	<0.004	<0.11	<0.034
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

Significance for days alive and free of organ dysfunction tested by Spearman's rho statistic.

Patients with sepsis (n=393) in the Protective-Protective Group had 28 day survival of 70.3 %, patients in the Risk-Protective Group had 28 day survival of 67 %, and patients in the Risk-Risk Group had 28 day survival of 56 % ($p < 0.04$ by Kaplan-Meier survival analysis over 28 days).

The organ dysfunction of patients who had sepsis according to group is shown in Table 11. There was a steady increase in organ dysfunction (scored as lower days alive and free of organ dysfunction and support) from Protective-Protective, through Risk-Protective to Risk-Risk groups.

Table 11. Days alive and free (DAF) of use of vasopressors, coagulation (platelets) dysfunction, INR, renal, cardiovascular dysfunction, hepatic dysfunction, SIRS 4 of 4, neurological (CNS) dysfunction, use of inotropic agents (inotropes), and renal support in 393 critically ill patients who had Sepsis according to group

PC 4732 C/EPCR 4054T Risk Group	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal	DAF CVS
Risk-Risk	15.6±11.1	17.7±11.1	17.2±11.4	14.7±11.8	13.4±11
Risk-	18.4±11.1	19.8±10.9	19±11.2	17.6±11.4	15.8±11

Protective					
Protective-Protective	19.3±10.4	21.2±10	19.7±10.2	19.4±11	17.6±10.5
P value	<0.007	<0.031	<0.036	<0.006	<0.055
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

PC 4732 C/EPCR 4054T Risk Group	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	DAF Inotropes
Risk-Risk	17.7±11.2	16.9±10.4	16.6±11.4	18.5±11.2
Risk-Protective	19.9±10.9	19.1±10.2	19.1±10.7	20.4±10.5
Protective-Protective	20.8±11	20.1±10	20.3±10.3	21.8±10
P value	<0.028	<0.007	<0.021	<0.013
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

PC 4732 C/EPCR 4054T Risk Group	DAF Renal Support
Risk-Risk	16.1±11.9
Risk-Protective	18.1±11.8
Protective-Protective	17.8±12.2
P value	< 0.09
	Mean±Standard Deviation

Significance for days alive and free of organ dysfunction tested by Spearman's rho statistic.

Patients with septic shock (n = 260) in the Protective-Protective Group had 28 day survival of 63 %, patients in the Risk-Protective Group had 28 day survival of 60 %, and

patients in the Risk-Risk Group had 28 day survival of 50 % ($p < 0.107$ by Kaplan-Meier survival analysis over 28 days).

We conclude that there is an interaction between risk alleles of protein C (4732C) and EPCR (4054T) (as defined above) that is associated with increased risks of death and multiple organ dysfunctions in systemic inflammatory response syndrome (SIRS), sepsis and septic shock.

EXAMPLE 4: Improved Response to Therapy with Activated Protein C (XIGRIS™)

Therapies for sepsis may include mechanical ventilation, support of circulation with vasopressors and inotropic agents, antibiotics, drainage of abscesses and surgery as appropriate. Activated protein C (APC or XIGRIS™ (when referring to APC as sold by Eli Lilly & Co., Indianapolis IN)) can improve survival of sepsis patients. The PROWESS trial showed that XIGRIS™ decreased 28 day mortality from 31 % (placebo) to 25 % (APC/XIGRIS™ – treated). XIGRIS™ was particularly effective in patients at high risk of death for example as identified by having an APACHE II score greater than or equal to 25. XIGRIS™ has been approved for treatment of severe sepsis at increased risk of death. In some jurisdictions, the high risk of death is identified as having an APACHE II score greater than or equal to 25; in other jurisdictions high risk of death is identified as having 2 or more organ dysfunctions or having an APACHE II score greater than or equal to 25.

All genotyping, clinical phenotyping, sample collection, sample analysis and statistical methods were performed as described herein. Patients with clinically defined sepsis, treated with activated protein C (XIGRIS™) and who had been genotyped for the protein C and EPCR polymorphisms were the subject of Example 4. A total of 893 patients were studied. Baseline characteristics and 28 day survival of each group (treated with XIGRIS™ and not treated with XIGRIS™) were determined and 38 patients were compared who had sepsis and were treated with XIGRIS™ to patients who had sepsis, but were not treated with XIGRIS™.

The severity of organ dysfunction that occurs in the setting of sepsis was also examined to determine whether XIGRIS™ reduces organ dysfunction in patients who have sepsis and

who have an at risk genotype of protein C or EPCR (protein C 2418 A, protein C 4732 C and EPCR 4054 T).

To determine the severity of organ dysfunction the Brussels scoring system was used. Each patient was assessed for each organ dysfunction on each day over 28 days after meeting at least two of four criteria for the systemic inflammatory response syndrome (SIRS). On each day, each organ system dysfunction was classified in each patient as being present (if organ dysfunction was moderate, severe or extreme according to the Brussels score) or absent (normal or mild according to the Brussels score). Days alive and free of organ dysfunction were calculated each day (from day 0 to day 28). Each day a patient had an organ dysfunction or was dead was scored as a zero for that organ dysfunction. In contrast, each day that a person was both alive and free of an organ dysfunction was scored as a one for that organ dysfunction. Then, the scores for each organ dysfunction of each patient were summed over the 28-day observation period. Thus, a score could range from 0 to 28. A higher score is beneficial because it indicates more days alive and free of organ dysfunction.

EXAMPLE 4A: Baseline Characteristics

The baseline characteristics of patients who had severe sepsis and who were treated with XIGRIS™ are shown in Table 12. These patients are typical of patients who have severe sepsis in that the average age was 54 years, the average APACHE II score was 31, there was frequent organ dysfunction and the survival was 58 percent.

TABLE 12. Baseline characteristics, survival and organ dysfunction (as days alive and free of organ dysfunction) of 38 patients who had sepsis and were treated with activated protein C (XIGRIS™).

	MEAN
AGE	54.1
APACHE II	31
SURGICAL (%)	26
SURVIVAL (% 28 DAY)	58
DAF ALI	12.1
DAF PRESSORS	15.1
DAF INOTROPES	17.5
DAF SIRS 4/4	15.4
ADMISSION SEPSIS (%)	100

ADMISSION SEPTIC SHOCK (%)	83
DAF STEROIDS	11.2
DAF CVS	13.2
DAF RESP	11
DAF PF 300	4
DAF VENTILATION	10.3
DAF CNS	15.8
DAF COAGULATION	15.9
DAF INR > 1.5	17.3
DAF RENAL	14.3
DAF RENAL SUPPORT	14.1
DAF HEPATIC	15.7

EXAMPLE 4B: Protein C 4732 Genotype

The effects of treatment with XIGRIS™ according to the genotype of patients for protein C 4732 T/C is shown in Table 13 below.

Table 13. Outcomes of patients who were treated with activated protein C (XIGRIS™) according to protein C 4732 genotype TT vs. CT, CC).

Protein C 4732 TT (non-risk genotype)	MEAN (n=18)	Protein C 4732 CT and CC (risk geneotype)	MEAN (n=10)
AGE	49.4	AGE	56.5
APACHE II	30	APACHE II	32
SURGICAL (%)	28	SURGICAL (%)	10
SURVIVAL (% 28 DAY)	61	SURVIVAL (% 28 DAY)	70 %
DAF ALI	11	DAF ALI	11.1
DAF PRESSORS	16.6	DAF PRESSORS	17.1
DAF INOTROPES	18.3	DAF INOTROPES	18.8
DAF SIRS 4/4	16.6	DAF SIRS 4/4	15.6
ADMISSION SEPSIS (%)	100	ADMISSION SEPSIS (%)	100%
ADMISSION SEPTIC SHOCK (%)	87	ADMISSION SEPTIC SHOCK (%)	86 %
DAF STEROIDS	13.4	DAF STEROIDS	12.6
DAF CVS	14.7	DAF CVS	14.9
DAF RESP	12.1	DAF RESP	11.7
DAF PF > 300	5.9	DAF PF > 300	2.9
DAF VENTILATION	11.3	DAF VENTILATION	11.2
DAF CNS	17.7	DAF CNS	14.5
DAF COAGULATION	17.7	DAF COAGULATION	16.8
DAF INR > 1.5	18.9	DAF INR > 1.5	20
DAF RENAL	15.9	DAF RENAL	16
DAF RENAL SUPPORT	15.3	DAF RENAL SUPPORT	12.1
DAF HEPATIC	17.6	DAF HEPATIC	16.9

Patients who had protein C 4732 CC and CT and were treated with XIGRIS™ had somewhat greater age and APACHE II score, than patients who were genotype protein C 4732 TT, yet the 4732 CC and CT patients had higher survival (70 percent compared to 61 percent) than patients who were genotype 4732 TT and treated with XIGRIS™.

Survival of patients who had sepsis and were not treated with XIGRIS™ were compared to patients who had sepsis and were treated with XIGRIS™ according to protein C 4732 genotype as shown in Table 14 below.

Table 14. Survival of patients who had severe sepsis who were treated/not treated with XIGRIS™ according to their genotype of protein C 4732.

Protein C 4732	Sepsis not treated with XIGRIS™ Survival (%)	Sepsis and treated with XIGRIS™ – Survival (%)	APACHE II of XIGRIS™-treated
4732 TT	68	61 N = 18	30
4732 CC, CT	57	70 N = 10	32

Patients who had the risk genotype (protein C 4732 CC, CT) and were not treated with XIGRIS™ had a survival of 57 percent, whereas treatment with XIGRIS™ increased survival of patients who were protein C 4732 CC, CT to 70 %. Thus, XIGRIS™ increased the absolute survival rate by 13 percent (70 percent – 57 percent = 13 percent) of patients who had the at risk genotype of protein C 4732.

Another way to examine the effect of XIGRIS™ on survival is to evaluate the relative increase in survival. XIGRIS™ increased the survival of patients who had severe sepsis who had the at risk genotype of protein C 4732 CC by 23 percent relatively (13/57 x 100) compared to protein C 4732 CT and TT. Another useful comparison to evaluate the effects of XIGRIS™ on survival according to protein C 4732 genotype is to compare the survival of protein C 4732 TT (wild type) in patients not treated with XIGRIS™ to patients who were genotype of protein C 4732 TT (wild type) and were treated with XIGRIS™. Of the patients who were not treated with XIGRIS™, the survival of wild type genotype of protein C 4732 (i.e. protein C 4732 TT) was higher than the risk genotype (protein C 4732 CC, CT) - 68 percent survival vs. 57 percent survival (wild type not treated with XIGRIS™). In contrast, the treatment with XIGRIS™ did not improve the survival of patients who had the wild type (non-risk) genotype (protein C 4732 TT) (61

percent survival if treated with XIGRIS™ vs. 68 percent survival if not treated with XIGRIS™). The treatment with XIGRIS™ increased the survival of patients who had the risk genotype (protein C 4732 CC, CT) such that their survival (70 percent) was higher than the survival of patients who had the wild type genotype of protein C 4732 who were treated with XIGRIS™ (70 percent vs. 67 percent).

EXAMPLE 4C: EPCR 4054 Genotype

As shown in Table 15, there was a slight increase in survival of patients who had the genotype EPCR 4054 TT who were treated with XIGRIS™ (70 percent survival) compared to patients who had the EPCR 4054 TT genotype who were not treated with XIGRIS™ (survival = 63 percent).

Table 15.Comparison of survival of patients with sepsis who were not treated with XIGRIS™ to patients with sepsis who were treated with XIGRIS™ according to EPCR 4054 genotype.

EPCR 4054	Sepsis survival not treated with XIGRIS™ (%)	Sepsis survival – treated with XIGRIS™ (%)	APACHE II
CC/CT	66	40 N = 5	37
TT	63	70 N = 23	29

EXAMPLE 4D: Combined Genotype of Protein C 4732 and EPCR 4054

XIGRIS™ had the greatest beneficial increase in survival in patients who had the combined risk – risk genotypes of protein C 4732 and EPCR 4054 (Table 16).

Table 16. Comparison of survival of patients with sepsis who were not treated with XIGRIS™ to patients with sepsis who were treated with XIGRIS™ according to protein C/EPCR genotype (protective – protective (non-risk – non-risk), risk – protective (risk – non-risk) pooled as "all others", risk – risk).

Protein C 4732 – EPCR 4054	Sepsis survival (%)	APC – treated survival (%)	APACHE II
All others	68	62 N = 21	30
Risk – Risk	58	83 N = 6	30

XIGRIS™ treatment increased survival of risk – risk compared to patients not treated with APC (58 % to 83 %), an absolute increase in survival of 25 percent. Another way to evaluate the effects of XIGRIS™ is to examine the relative increase in survival. XIGRIS™ increased the relative chance of survival of the risk – risk genotype of protein C 4732 and EPCR 4054 by 43 percent (25/58 x 100).

Of the patients who had sepsis and received XIGRIS™ the protective – protective and risk – protective (werein protective = non-risk) genotypes of protein C 4732 and EPCR 4054 were pooled because of the small number of patients in the protective – protective group. The survival of the pooled protective – protective and risk – protective groups who received XIGRIS™ was 62 percent; in contrast, the survival of the patients who had sepsis who had the risk – risk combined genotype who received XIGRIS™ was higher at 83 percent. Thus, in another useful comparison, XIGRIS™ treatment increased the survival of sepsis patients having the risk – risk genotype of protein C 4732 and EPCR 4054, such that it exceeded the survival of the pooled protective – protective and risk – protective groups who received XIGRIS™ by 21 percent (62 percent vs. 83 percent). XIGRIS™ increased the survival of patients who had the risk – risk combined genotype of protein C 4732 C and EPCR 4054 T.

XIGRIS™ also increased survival of patients who had the risk – protective haplotypes compared to risk – protective patients who were not treated with XIGRIS™ from 67 to 71 %, an absolute increase in survival of 4 percent, and a relative increase in survival of 6 percent.

EXAMPLE 4E: SUMMARY OF EFFECTS OF XIGRIS™ ON PROTEIN C 2418, PROTEIN C 4732, EPCR 4054 AND THE COMBINED PROTEIN C 4732/EPCR 4054 GENOTYPES

Table 17 summarizes the effects of XIGRIS™ on survival of patients according to the protein C 2418, protein C 4732, EPCR 4054 and the combined protein C 4732/EPCR 4054 genotypes.

Table 17. Summary of effects of XIGRIS™ on protein C 2418, protein C 4732, EPCR 4054 and the combined protein C 4732/EPCR 4054 genotypes.

Genotype	Sepsis survival – Not treated with XIGRIS™	Sepsis survival – Treated with XIGRIS™	Absolute Increase in Survival with XIGRIS™	Relative Increase in Survival with XIGRIS™
EPCR 4054 TT	63 %	70 %	7 %	10%
Protein C 2418 AA	58 %	64 %	6 %	10 %
Protein C 4732 CC/CT	57 %	70 %	13 %	23 %
Protein C 4732 CC/ EPCR 4054 TT (Risk – Risk)	58 %	83 %	25 %	43 %

There was an internally consistent graded absolute increase in survival prediction according to the EPCR 4054, protein C 2418, protein C 4732, 4054 and the combined protein C 4732/EPCR 4054 genotypes and a useful prediction of benefit from treatment with XIGRIS™.

XIGRIS™ provided a graded increase in survival according to genotype. For example, XIGRIS™ provided an absolute increase in survival of 7 percent for the EPCR 4054 TT, 6 percent for the protein C 2418 AA, 13 percent for the protein C 4732 CC, CT, and 25 percent for the protein C 4732 CC, CT and EPCR 4054 TT combined risk – risk genotype.

EXAMPLE 4F: Effects of Treatment with XIGRIS™ on Organ Dysfunction According to Protein C 4732 Genotype

Table 18 shows that patients who had the risk – risk genotype of Protein C 4732 C/EPCR 4054 T who were treated with XIGRIS™ had less organ dysfunction as shown by more days alive and free of organ dysfunction and organ support than did patients who had the protein C 4732 C/EPR 4054 T risk – risk genotype who had sepsis and who were not treated with XIGRIS™. In particular, patients treated with XIGRIS™ had less need for vasopressors (more DAF Pressors), less need for inotropes (more DAF inotropes), less cardiovascular dysfunction (more DAF Cardiovascular), less respiratory dysfunction (more DAF Respiratory), less need for ventilation (more DAF Ventilation), less coagulopathy (more DAF INR > 1.5), and less hepatic dysfunction (more DAF hepatic).

Table 18. Comparison of organ dysfunction scored as days alive and free (DAF) of organ dysfunction and organ support of patients who had sepsis who were not treated with XIGRIS™ to patients who had sepsis who were treated with XIGRIS™ according to protein C 4732 C genotype/EPCR 4054 T genotype in the risk – risk subgroup (as defined below in detailed patent) .

Protein C 4732 C – EPCR 4054 T Risk - Risk	Sepsis not treated with XIGRIS™	XIGRIS™-treated N = 6
Age	58	56
APACHE II	25	30
DAF Pressors	15.6	19.5
DAF Inotropes	18.5	21.8
DAF Cardiovascular	13.9	16.5
DAF Respiratory	11.4	15
DAF Ventilation	10.4	14.3
DAF CNS	16.6	16.5
DAF INR > 1.5	17.2	23.7
DAF Renal	14.7	14
DAF Hepatic	17.7	18.5

Clinical Implications

Patients with sepsis, severe sepsis or SIRS should be genotyped to assess their protein C 2418, protein C 4732 and EPCR 4054 genotypes or the genotypes of polymorphism sites in linkage disequilibrium with protein C 2418, protein C 4732 and EPCR 4054. Patients could then be classified by genotype into a risk category regarding their unique risk of death by genotype. Furthermore, the patient's genotype can be used to also determine how well they will respond to activated protein C (XIGRIS™) or other anti-inflammatory agents or anti-coagulant agents. It was found that there was a clear graded increase in absolute survival when XIGRIS™ was used as treatment according to the genotype of the patient for EPCR 4054, protein C 2418, protein C 4732, and the combined risk – risk genotype of protein C 4732/EPCR 4054. Thus, clinicians can now administer XIGRIS™ according to a patient-tailored risk assessment. Each patient's unique genotype of protein C and EPCR can be used to make a unique assessment of risk of death and to predict whether or not a patient is likely to benefit from XIGRIS™ treatment.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of the teachings of this invention that changes and modification

may be made thereto without departing from the spirit or scope of the appended claims. All patents, patent applications and publications referred to herein are hereby incorporated by reference.

CLAIMS

What is Claimed is:

1. A method for obtaining a prognosis for a patient having or at risk of developing an inflammatory condition, the method comprising determining a genotype including one or more polymorphism sites in the protein C gene; EPCR gene or a combination thereof for the patient, wherein said genotype is indicative of an ability of the patient to recover from the inflammatory condition.

2. The method of claim 1, wherein a polymorphism site corresponds to position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2.

3. The method of claim 1, wherein polymorphism sites from both the Protein C gene and EPCR gene are combined, wherein said polymorphism sites correspond to one or more of position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or position 2418 of SEQ ID NO:1; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2 or position 2418 of SEQ ID NO:1.

4. The method of claim 2 or 3, wherein the polymorphism site in linkage disequilibrium with position 4732 corresponds to position 4813, 6379 or 6762 in SEQ ID NO: 1.

5. The method of claim 2 or 3, wherein the polymorphism site in linkage

disequilibrium with position 4054 corresponds to position 2973, 3063, 3402, 4946, 5515 or 6196 in SEQ ID NO: 2.

6. The method of claim 3, wherein the polymorphism site in linkage disequilibrium with position 2418 corresponds to position 1386, 2583 or 3920 in SEQ ID NO: 1.

7. The method of claim 2 or 3, wherein the polymorphism site in linkage disequilibrium with position 4732 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

9198 and 5867;
9198 and 4800;
3220 and 5867; and
3220 and 4800.

8. The method of claim 3, wherein the polymorphism site in linkage disequilibrium with position 2418 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

5867 and 2405;
5867 and 4919;
5867 and 4956;
5867 and 6187;
5867 and 9534;
5867 and 12109;
4800 and 2405;
4800 and 4919;
4800 and 4956;
4800 and 6187;
4800 and 9534;
4800 and 12109;
9198 and 6379 and 2405;
9198 and 6379 and 4919;

9198 and 6379 and 4956;
9198 and 6379 and 6187;
9198 and 6379 and 9534; and
9198 and 6379 and 12109.

9. The method of any one of claims 1-8, further comprising comparing the genotype so determined with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition.

10. The method any one of claims 1-9, further comprising obtaining protein C gene or EPCR gene sequence information for the patient.

11. The method any one of claims 1-9, wherein said determining of genotype is performed on a nucleic acid sample from the patient.

12. The method of claim 11, further comprising obtaining a nucleic acid sample from the patient.

13. The method any one of claims 1-12, wherein said determining of genotype comprises one or more of:

(a) restriction fragment length analysis;

(b) sequencing;

(c) hybridization;

(d) oligonucleotide ligation assay;

(e) ligation rolling circle amplification;

(f) 5′ nuclease assay;

(g) polymerase proofreading methods;

(h) allele specific PCR; and

(i) reading sequence data.

14. The method of any one of claims 1-13, wherein the genotype of the patient is indicative of a decreased likelihood of recovery from an inflammatory condition or an increased risk of having a poor outcome.

15. The method of claim 14, wherein the prognosis is indicative of severe cardiovascular or respiratory dysfunction in critically ill patients.

16. The method of claim 14 or 15, wherein the genotype is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 C;
4813 A;
6379 G;
6762 A;
9198 C and 5867 A;
9198 C and 4800 G;
3220 A and 5867 A; and
3220 A and 4800 G

or

1386 T;
2418 A;
2583 A;
3920 T;
5867 A and 2405 T;
5867 A and 4919 A;
5867 A and 4956 T;
5867 A and 6187 C;
5867 A and 9534 T;
5867 A and 12109 T;

4800 G and 2405 T;
4800 G and 4919 A;
4800 G and 4956 T;
4800 G and 6187 C;
4800 G and 9534 T;
4800 G and 12109 T;
9198 A and 6379 G and 2405 T;
9198 A and 6379 G and 4919 A;
9198 A and 6379 G and 4956 T;
9198 A and 6379 G and 6187 C;
9198 A and 6379 G and 9534 T; and
9198 A and 6379 G and 12109 T.

17. The method of claim 14 or 15, wherein the genotype is selected from the group of EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

6196 G;
5515 T;
4946 T;
4054 T;
3402 G;
3063 G; and
2973 C.

18. The method of any one of claims 1-13, wherein the genotype of the patient is indicative of an increased likelihood of recovery from an inflammatory condition.

19. The method of claim 18, wherein the prognosis is indicative of less severe cardiovascular or respiratory dysfunction in critically ill patients.

20. The method of claim 18 or 19, wherein the genotype is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 T;
4813 G;
6379 A;

6762 G;
9198 A and 5867 G;
9198 A and 4800 C;
3220 G and 5867 G; and
3220 G and 4800 C

or

1386 C;
2418 G;
2583 T;
3920 C;
5867 G and 2405 C;
5867 G and 4919 G;
5867 G and 4956 C;
5867 G and 6187 T;
5867 G and 9534 C;
5867 G and 12109 C;
4800 C and 2405 C;
4800 C and 4919 G;
4800 C and 4956 C;
4800 C and 6187 T;
4800 C and 9534 C; and
4800 C and 12109 C.

21. The method of claim 18 or 19, wherein the genotype is selected from the group of EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

6196 C;
5515 C;
4946 C;
4054 C;
3402 C;
3063 A; and
2973 T.

22. The method of any one of claims 1-21, wherein the inflammatory condition is selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumanitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to

trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglotittis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus

erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

23. The method of any one of claims 1-22, wherein the inflammatory condition is systemic inflammatory response syndrome.

24. A method of identifying a polymorphism in a protein C gene sequence that correlates with a patient prognosis, the method comprising:

a) obtaining protein C gene or EPCR sequence information from a group of patients;

b) identifying a site of at least one polymorphism in the protein C or EPCR gene;

c) determining genotypes at the site for individual patients in the group;

d) determining an ability of individual patients in the group to recover from the inflammatory condition; and

e) correlating genotypes determined at step (c) with patient abilities determined at step (d).

25. The method of claim 24, wherein the inflammatory condition is selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome

(ARDS), acute lung injury, aspiration pneumanitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglotittis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP Syndrome, mycobacterialtuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and

autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

26. A kit for determining a genotype at a defined nucleotide position within a polymorphism site in a protein C gene or an EPCR gene from a patient to provide a prognosis of the patient's ability to recover from an inflammatory condition, the kit comprising, a restriction enzyme capable of distinguishing alternate nucleotides at the polymorphism site or a labeled oligonucleotide having sufficient complementary to the polymorphism site and capable of distinguishing said alternate nucleotides.

27. The kit of claim 26, wherein a polymorphism site corresponds to position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2.

28. The kit of claim 26, wherein the kit is suitable for determining genotype at one or more nucleotide positions within each of the protein C gene or the EPCR gene, wherein said polymorphism sites correspond to one or more of position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or position 2418 of SEQ ID

NO:1; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2 or position 2418 of SEQ ID NO:1.

29. The kit of claim 26, 27 or 28 further comprising an oligonucleotide or a set of oligonucleotides suitable to amplify a region including the polymorphism site.

30. The kit of claim 29, further comprising a polymerization agent.

31. The kit of any one of claims 26-30, further comprising instructions for using the kit to determine genotype.

32. A method for selecting a group of patients for determining the efficacy of a candidate drug known or suspected of being useful for the treatment of an inflammatory condition, the method comprising determining a genotype for one or more polymorphism sites in the protein C gene or EPCR gene for each patient, wherein said genotype is indicative of the patient's ability to recover from the inflammatory condition and sorting patients based on their genotype.

33. The method of claim 32 further comprising, administering the candidate drug to the patients or a subset of patients and determining each patient's ability to recover from the inflammatory condition.

34. The method of claim 33, further comprising comparing patient response to the candidate drug based on genotype of the patient.

35. A method of treating an inflammatory condition in a patient in need thereof, the method comprising administering to the patient an anti-inflammatory agent or an anti-coagulant agent, wherein said patient has a protein C gene or EPCR gene risk genotype.

36. A method of treating an inflammatory condition in a patient in need thereof, the method comprising:

(a) selecting a patient having a risk genotype in their protein C gene or EPCR gene; and

(b) administering to said patient an anti-inflammatory agent or an anti-coagulant agent.

37. A method of treating an inflammatory condition in a mammal in need thereof, the method comprising administering to the mammal an anti-inflammatory agent or an anti-coagulant agent, wherein said mammal has a protein C gene or EPCR gene risk genotype.

38. A method of treating an inflammatory condition in a mammal, the method comprising:

(a) selecting a mammal having a risk genotype in their protein C gene or EPCR gene; and

(b) administering an anti-inflammatory agent or an anti-coagulant agent to the selected mammal.

39. A method of selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, comprising the step of identifying a subject having one or more risk genotypes in their protein C gene or EPCR gene, wherein the identification of a subject with one or more risk genotypes is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

40. A method of treating a subject with an inflammatory condition by administering an anti-inflammatory agent or an anti-coagulant agent, the method comprising administering the anti-inflammatory agent or the anti-coagulant agent to subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant

agent.

41. A method of identifying a subject with increased responsiveness to treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, comprising the step of screening a population of subjects to identify those subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with a risk genotype in their protein C gene or EPCR gene is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

42. A method of selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, comprising the step of identifying a subject having a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

43. A method of treating an inflammatory condition in a patient, the method comprising administering an anti-inflammatory agent or an anti-coagulant agent to the patient, wherein said patient has a risk genotype in their protein C gene or EPCR gene.

44. A method of treating an inflammatory condition in a patient, the method comprising:

(a) identifying a patient having a risk genotype in their protein C gene or EPCR gene; and

(b) administering an anti-inflammatory agent or an anti-coagulant agent to the patient.

45. The use of an anti-inflammatory agent or an anti-coagulant in the manufacture of a medicament for the treatment of an inflammatory condition, wherein the patients treated have a risk genotype in their protein C gene or EPCR gene.

46. The use of an anti-inflammatory agent or an anti-coagulant in the manufacture of a medicament for the treatment of an inflammatory condition in a subset of patients, wherein the subset of patients have a risk genotype in their protein C gene or EPCR gene.

47. The method of any one of claims 35 to 44, further comprising determining the patients APACHE II score as an assessment of patient risk.

48. The method of any one of claims 35 to 44 and 47, further comprising determining the number of organ system failures for the patient as an assessment of patient risk.

49. The method of claim 48, wherein the patients APACHE II score is indicative of increased risk when ≥ 25.

50. The method of claim 49, wherein 2 or more organ system failures are indicative of increased patient risk.

51. The method of any one of claims 35 to 50, wherein the inflammatory condition is selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumanitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis,

patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglotittis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

52. The method of any one of claims 35-51, wherein the inflammatory condition is systemic inflammatory response syndrome.

53. The method of any one of claims 35-52, wherein the risk genotype is located at a polymorphism site corresponding to position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or a polymorphism site in linkage disequilibrium with 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2.

54. The method of any one of claims 35-52, wherein the risk genotype is located at a polymorphism site corresponding to position 2418 of SEQ ID NO:1 or a polymorphism site in linkage disequilibrium thereto.

55. The method of any one of claims 35-52, wherein the risk genotypes from the Protein C gene and EPCR gene are located at polymorphism sites corresponding to one or more of position 4732 of SEQ ID NO:1; position 4054 of SEQ ID NO:2; position 2418 of SEQ ID NO:1; and a polymorphism site in linkage disequilibrium with 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2 or position 2418 of SEQ ID NO:1.

56. The method of claim 53 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 4732 corresponds to position 4813, 6379 or 6762 in SEQ ID NO: 1.

57. The method of claim 53 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 4054 corresponds to position 2973, 3063, 3402, 4946, 5515 or 6196 in SEQ ID NO: 2.

58. The method of claim 54 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 2418 corresponds to position 1386, 2583 or 3920 in SEQ ID NO: 1.

59. The method of claim 53 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 4732 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

9198 and 5867;
9198 and 4800;
3220 and 5867; and

3220 and 4800.

60. The method of claim 54 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 2418 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

5867 and 2405;
5867 and 4919;
5867 and 4956;
5867 and 6187;
5867 and 9534;
5867 and 12109;
4800 and 2405;
4800 and 4919;
4800 and 4956;
4800 and 6187;
4800 and 9534;
4800 and 12109;
9198 and 6379 and 2405;
9198 and 6379 and 4919;
9198 and 6379 and 4956;
9198 and 6379 and 6187;
9198 and 6379 and 9534; and
9198 and 6379 and 12109.

61. The method of any one of claims 53, 54, 55, 56, 58, 59 or 60, wherein the genotype for an increased risk or risk genotype is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 C;
4813 A;
6379 G;
6762 A;
9198 C and 5867 A;
9198 C and 4800 G;
3220 A and 5867 A; and
3220 A and 4800 G

or

1386 T;
2418 A;
2583 A;
3920 T;
5867 A and 2405 T;
5867 A and 4919 A;
5867 A and 4956 T;
5867 A and 6187 C;
5867 A and 9534 T;
5867 A and 12109 T;
4800 G and 2405 T;
4800 G and 4919 A;
4800 G and 4956 T;
4800 G and 6187 C;
4800 G and 9534 T;
4800 G and 12109 T;
9198 A and 6379 G and 2405 T;
9198 A and 6379 G and 4919 A;
9198 A and 6379 G and 4956 T;
9198 A and 6379 G and 6187 C;
9198 A and 6379 G and 9534 T; and
9198 A and 6379 G and 12109 T.

62. The method of any one of claims 53, 55 or 57, wherein the genotype for an increased risk or risk genotype is selected from the group of EPCR single polymorphism sites and combined polymorphism sites in SEQ ID NO: 2 consisting of:

6196 G;
5515 T;
4946 T;
4054 T;
3402 G;
3063 G; and
2973 C.

63. The method of any one of claims 61 or 62, wherein the genotype of the patient is indicative of an increased risk of poor outcome from an inflammatory condition.

64. The method of any one of claims 61, 62 or 63, wherein the patient having an increased risk of poor outcome from an inflammatory condition is preferentially selected for administration the anti-inflammatory agent or the anti-coagulant agent.

65. The method of any one of claims 53, 54, 55, 56, 58, 59 or 60, wherein the genotype for a decreased risk is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 T;
4813 G;
6379 A;
6762 G;
9198 A and 5867 G;
9198 A and 4800 C;
3220 G and 5867 G; and
3220 G and 4800 C

or

1386 C;
2418 G;
2583 T;
3920 C;
5867 G and 2405 C;
5867 G and 4919 G;
5867 G and 4956 C;
5867 G and 6187 T;
5867 G and 9534 C;
5867 G and 12109 C;
4800 C and 2405 C;
4800 C and 4919 G;
4800 C and 4956 C;
4800 C and 6187 T;
4800 C and 9534 C; and
4800 C and 12109 C.

66. The method of any one of claims 53, 55 or 57, wherein the genotype for a decreased risk is selected from the group of EPCR single polymorphism sites and

combined polymorphism sites in SEQ ID NO: 2 consisting of:

6196 C;
5515 C;
4946 C;
4054 C;
3402 C;
3063 A; and
2973 T.

67. The method of any one of claims 65 or 66, wherein the genotype of the patient is indicative of a decreased risk of poor outcome from an inflammatory condition.

68. The method of any one of claims 65, 66 or 67, wherein the patient having a decreased risk of poor outcome from an inflammatory condition is preferentially not selected for administration the anti-inflammatory agent or the anti-coagulant agent.

69. The method of any one claims 35 to 67, wherein the anti-inflammatory agent or the anti-coagulant agent is selected from any one or more of the following:

(a) activated protein C;

(b) tissue factor pathway inhibitors;

(c) platelet activating factor hydrolase;

(d) PAF-AH enzyme analogues;

(e) antibody to tumor necrosis factor alpha;

(f) soluble tumor necrosis factor receptor-immunoglobulin G1;

(g) procysteine;

(h) elastase inhibitor;

(i) human recombinant interlukin 1 receptor antagonists; and

(j) antibodies, inhibitors and antagonists to endotoxin, tumour necrosis factor receptor, interleukin-6, high mobility group box, tissue plasminogen activator, bradykinin, CD-14 and interleukin-10.

70. The method of any one claims 35 to 69, wherein the anti-inflammatory agent or the anti-coagulant agent is activated protein C.

71. The method of any one claims 35 to 70, wherein the anti-inflammatory agent or the anti-coagulant agent is drotecogin alfa activated.

ABSTRACT

The invention provides methods and kits for obtaining a prognosis for a patient having or at risk of developing an inflammatory condition and for identifying patients having a greater benefit from treatment with an anti-inflammatory agent or an anti-coagulant agent. The method generally comprises determining a protein C and/or EPCR genotype(s) of a patient for a polymorphisms in the these genes, comparing the determined genotype with known genotypes for the polymorphism that correspond with the ability of the patient to recover from the inflammatory condition and identifying patients based on their prognosis. The invention also provides for methods of identifying potential patients having an inflammatory condition who are more likely to benefit from treatment with an anti-inflammatory agent or anti-coagulant agent and subsequent to treatment recover from the inflammatory condition. The invention also provides for methods of treating such patients with an anti-inflammatory agent or anti-coagulant agent based on the patient's geneotype.

FIGURE 1

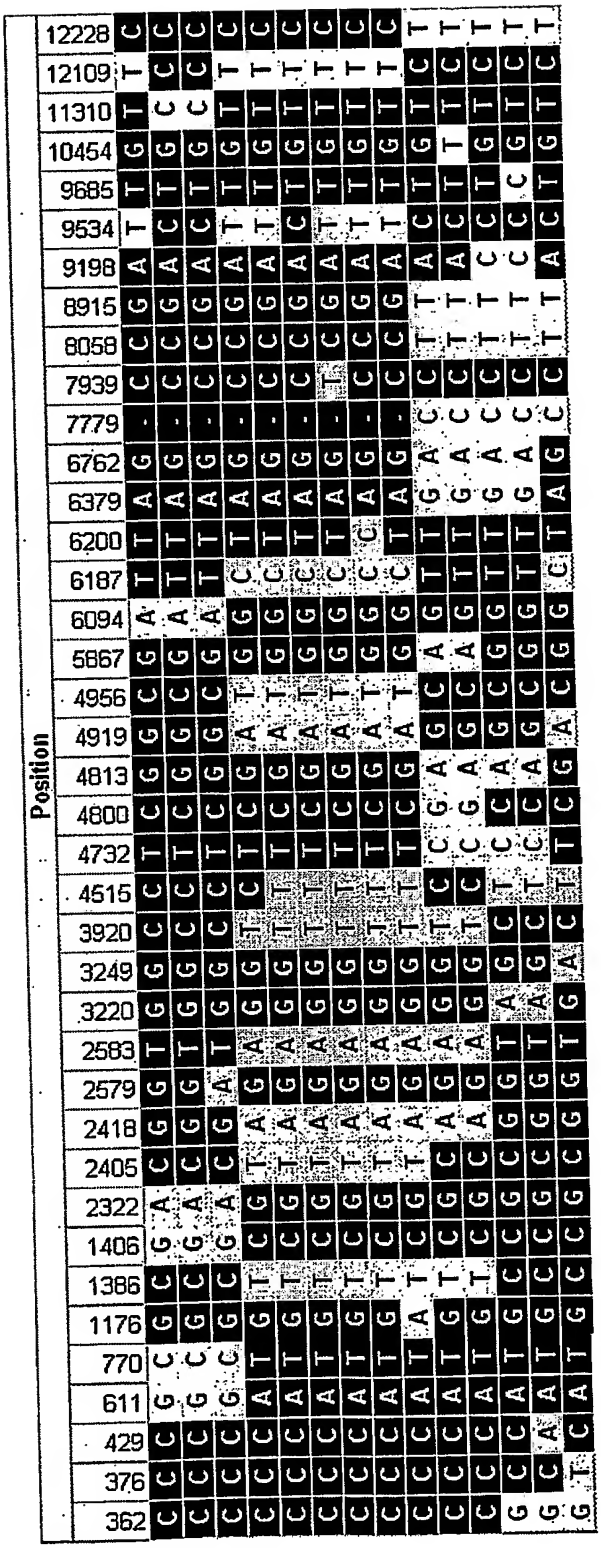

FIGURE 2

Position													
837	1940	2313	2973	3063	3402	3514	3600	4054	4946	5329	5515	6118	6196
C	G	A	C	G	G	G	T	T	T	G	T	A	G
C	A	A	C	G	G	G	T	T	T	G	T	A	G
C	G	A	C	G	G	A	T	T	T	G	T	A	G
C	G	A	C	G	G	G	C	T	T	A	T	A	G
G	G	A	C	G	G	G	T	T	T	G	T	G	G
C	G	A	T	A	C	G	T	C	C	G	C	A	C
C	G	C	T	A	C	G	T	C	C	G	C	A	C

FIGURE 3

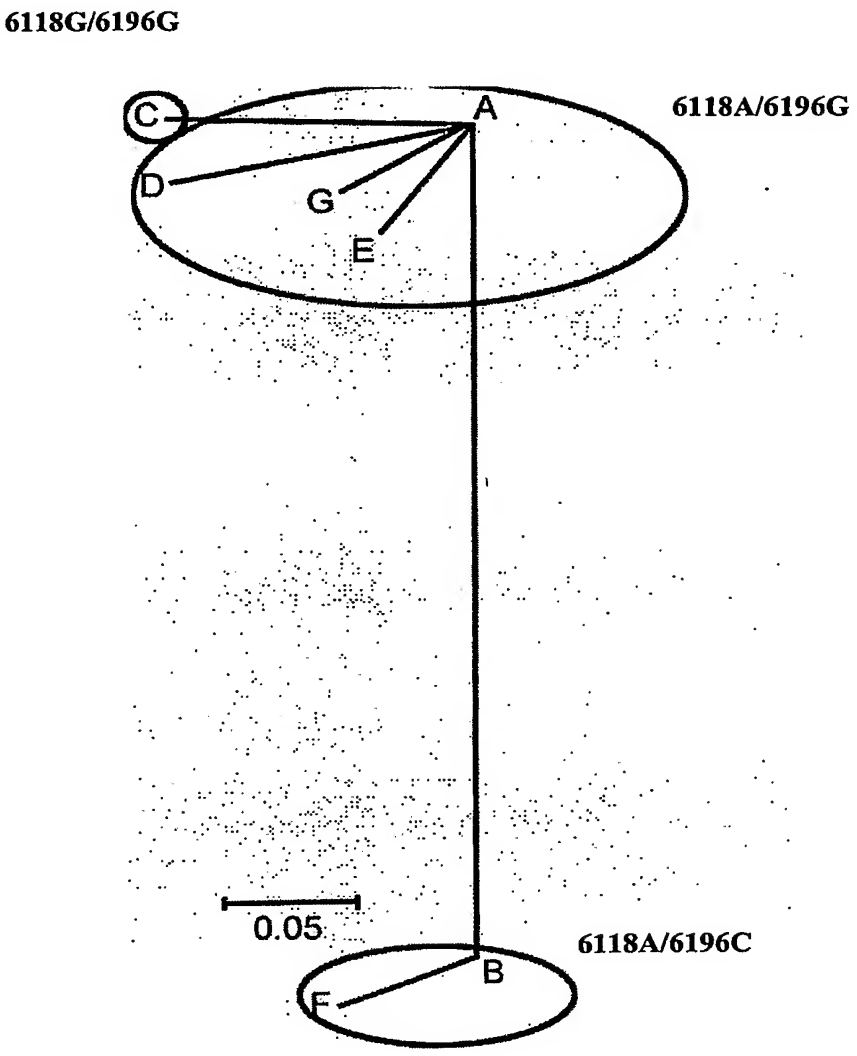

FIGURE 4

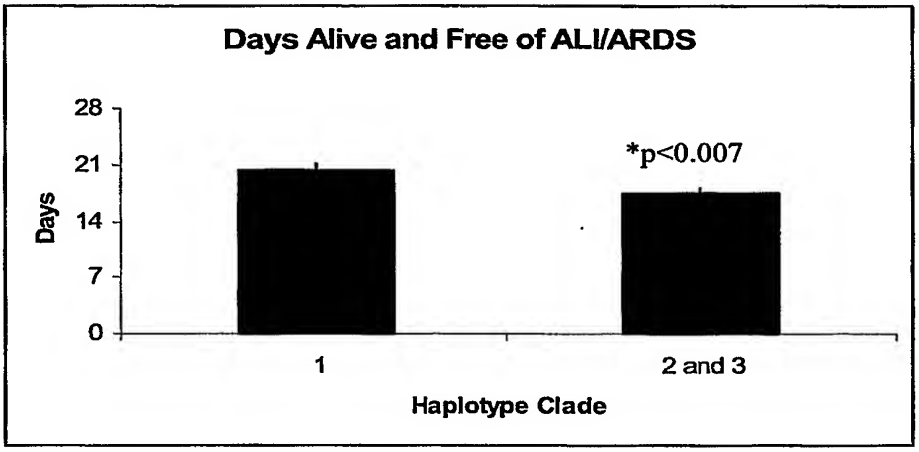

FIGURE 5